

Update on HPV vaccination and HPV screening

Quadrivalent vaccine also protects against HPV-related vulval and vaginal lesions, and an HPV DNA screening test proves to be more reliable than Pap testing.

What's new, what's important

Even though the incidence of cancer of the cervix is not that widespread in the Western world, about half a million women will be diagnosed with cancer of the cervix worldwide. It is the leading or second leading cause of mortality in many developing countries.

Discovery of the human papilloma virus (HPV), coupled with the pursuit of vaccination for cancer prevention, is one of the most impressive stories in modern medicine. The 2008 Nobel Prize in Physiology or Medicine was given to Prof. Harald zur Hausen of Germany for his part in establishing the role of HPV in the etiology of cervical cancer.

As experts in the treatment of cancer, we should be knowledgeable about the recommendations and controversies regarding HPV vaccination. The American Cancer Society recommends HPV vaccination for girls 11–12 years old and 13–18 years old to catch up on missed vaccines or to complete the series.

Pap smear and high-risk HPV (HRHPV) testing remain important parts of cervical cancer screening of women, and many other tests are evolving.

—Jame Abraham, MD
Section Editor

Infection with human papilloma-virus (HPV) types 16 and 18 is associated with cervical high-grade intraepithelial neoplasia and adenocarcinoma, as well as vulvar and vaginal high-grade intraepithelial neoplasias and adenocarcinomas. HPV vaccines have been shown to be effective in preventing HPV-related cervical intraepithelial neoplasia (CIN) and adenocarcinoma in situ, and a quadrivalent HPV 6/11/16/18 vaccine (Gardasil) also has been found to protect against HPV infection due to types 6 and 11 (anogenital warts and low-grade neoplasias). Recent studies indicate that the quadrivalent HPV vaccine is protective against HPV 16- and 18-related

high-grade vulval and vaginal lesions,¹ suggesting that it may prove protective against HPV-related cancers in these areas. Another recent study indicates that HPV DNA screening has greater sensitivity for detecting cervical intraepithelial neoplasia than Papanicolaou (Pap) testing.²

Broadened applications for quadrivalent HPV vaccine

A placebo-controlled trial of a three-dose regimen of HPV 16 virus-like-particle vaccine in 2,392 women aged 16–23 years, reported in 2002,³ showed that the rate of persistent HPV 16 infection over a median follow-up of 17 months was 0 versus 3.8

per 100 woman-years among females given a placebo. All nine cases of HPV 16-related CIN occurred in placebo recipients. Since then, the quadrivalent HPV 6/11/16/18 L1 viruslike-particle vaccine has been shown to be 99% effective against HPV 16- or 18-related CIN (grade 2/3) and cervical adenocarcinoma in situ.⁴

A recent combined analysis of three randomized placebo-controlled trials⁴ examined the efficacy of this quadrivalent vaccine in protecting against high-grade vulval and vaginal lesions, which are precursors to invasive cancer in these areas.¹ In total, 18,174 girls and women aged 16–26 years received three injections of vaccine or placebo (day 1, month 2, and month 6) and underwent a detailed anogenital examination on day 1, 1 month after the last dose, and at 6- to 12-month intervals thereafter for up to 48 months. The primary endpoint was the combined incidence of HPV 16- or 18-associated high-grade vulval (VIN2/3) or vaginal (VaIN2/3) intraepithelial neoplasia. Mean follow-up was 3 years. The per-protocol analysis included women without evidence of HPV 16 or 18 infection through 1 month after the third study dose (7,811 vaccine recipients and 7,785 placebo recipients).

On this analysis, histologically confirmed HPV 16- or 18-associated VIN2/3 or VaIN2/3 occurred in 15 women given placebo and none of those who received the vaccine, yielding a vaccine efficacy of 100% (95%

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TABLE 1

HPV vaccine efficacy in preventing vulval and vaginal HPV 16/18-related high-grade lesions and all high-grade lesions (irrespective of cause)

Population	Cases/number of women (rate: cases/100 person-years at risk)		Vaccine efficacy (95% CI)
	Vaccine (n = 9,087)	Placebo (n = 9,087)	
<i>Per-protocol susceptible population^a</i>			
VIN2/3 or VaIN2/3			
HPV 16- or 18-related	0/7,811 (0.00)	15/7,785 (0.08)	100% (72% to 100%)
HPV 16-related	0/6,687 (0.00)	13/6,500 (0.08)	100% (68% to 100%)
HPV 18-related	0/7,450 (0.00)	2/7,381 (0.01)	100% (-427% to 100%)
VIN2/3			
HPV 16- or 18-related	0/7,811 (0.00)	8/7,785 (0.04)	100% (42% to 100%)
HPV 16-related	0/6,687 (0.00)	7/6,500 (0.04)	100% (33% to 100%)
HPV 18-related	0/7,450 (0.00)	1/7,381 (0.01)	100% (<-999% to 100%)
VaIN2/3			
HPV 16- or 18-related	0/7,811 (0.00)	7/7,785 (0.04)	100% (31% to 100%)
HPV 16-related	0/6,687 (0.00)	6/6,500 (0.04)	100% (18% to 100%)
HPV 18-related	0/7,450 (0.00)	1/7,381 (0.01)	100% (<-999% to 100%)
<i>Intent-to-treat population^b</i>			
VIN2/3 or VaIN2/3			
HPV 16- or 18-related	9/9,087 (0.03)	31/9,087 (0.12)	71% (37% to 88%)
HPV 16-related	8/9,087 (0.03)	29/9,087 (0.11)	72% (38% to 89%)
HPV 18-related	1/9,087 (0.00)	3/9,087 (0.01)	67% (-316% to 99%)
VIN2/3			
HPV 16- or 18-related	8/9,087 (0.03)	21/9,087 (0.08)	62% (10% to 85%)
HPV 16-related	8/9,087 (0.03)	21/9,087 (0.08)	62% (10% to 85%)
HPV 18-related	0/9,087 (0.03)	1/9,087 (0.00)	100% (<-999% to 100%)
VaIN2/3			
HPV 16- or 18-related	2/9,087 (0.01)	11/9,087 (0.04)	82% (17% to 98%)
HPV 16-related	1/9,087 (0.00)	9/9,087 (0.03)	89% (20% to 100%)
HPV 18-related	1/9,087 (0.00)	2/9,087 (0.01)	50% (-863% to 99%)
<i>All high-grade lesions, intent-to-treat population^b</i>			
All VIN2/3 or VaIN2/3	27/9,087 (0.10)	53/9,087 (0.20)	49% (18% to 69%)
All VIN2/3	16/9,087 (0.06)	33/9,087 (0.12)	51% (9% to 75%)
All VaIN2/3	12/9,087 (0.05)	21/9,087 (0.08)	43% (-22% to 74%)

VIN2/3 = vulva intraepithelial neoplasia; VaIN2/3 = vaginal intraepithelial neoplasia; HPV = human papillomavirus; CI = confidence interval

^a Includes women who were HPV DNA-negative by polymerase chain reaction (PCR) and seronegative for the relevant vaccine-HPV type at enrollment, remained PCR negative for the same HPV type through 1 month after receiving the third vaccine dose, and received three vaccine doses within 1 year

^b Includes women with prevalent anogenital disease and infections due to any high- or low-risk HPV type before vaccination

Source: Joura et al¹

confidence interval [CI], 72%–100%). Among all women (intent-to-treat population, including those who might have been infected when the trial started), HPV 16- or 18-associated VIN2/3 or VaIN2/3 cases occurred in 9 of 9,087 vaccine recipients (0.10%) and 31 of

9,087 placebo recipients (0.34%), yielding a vaccine efficacy of 71% (95% CI, 37%–88%). Cases of VIN2/3 or VaIN2/3 irrespective of whether HPV DNA was detected in the lesion occurred in 27 vaccine recipients and 53 placebo recipients, representing an effi-

cacy of 49% (95% CI, 18%–69%). Efficacy rates according to HPV type, type of lesion, and predefined study population are shown in Table 1. The data indicate that the quadrivalent vaccine is highly effective in preventing HPV 16- and HPV 18-related high-grade vulval and vaginal lesions, which could result in reduced rates of HPV-related vulval and vaginal cancers.

HPV DNA screening vs Pap testing

Currently, DNA testing for oncogenic HPV types is used in clinical practice mainly to decide on colposcopy for women with atypical squamous cells of undetermined significance (ASCUS) on Pap testing. However, one study indicates that CIN can be more reliably detected by HPV DNA screening.² In this study, 10,154 Canadian women aged 30–69 years underwent testing for high-grade CIN with both a US Food and Drug Administration-approved HPV DNA test (*digene* Hybrid Capture 2; QIAGEN, Germantown, Md) and a conventional Pap test; both tests were performed in random sequence at the same clinical visit. Positive test results were defined as ASCUS or worse on Pap testing and an HPV DNA level of ≥ 1 pg/mL on HPV testing. Colposcopy and biopsy were performed in women with positive test results and in a random sample of women with negative results. Findings in the latter group were used to correct for verification bias, because verification of lesions only in patients with positive results can lead to an overestimation of test sensitivity.

Overall, 723 of 795 women with positive CIN test results and 665 of 9,359 women with negative results underwent colposcopy. Cases were defined based upon conservative or liberal criteria. The conservative definition required confirmation by a loop electrosurgical excision procedure (LEEP) or, if ablative treatment was used, by confirmatory biopsy. The liberal definition included all cases of grade 2/3

CIN, adenocarcinoma in situ, or cervical cancer confirmed by histologic examination of any of the ectocervical or endocervical biopsy specimens.

On the conservative definition, with correction for verification bias, the sensitivity in detecting grade 2/3 CIN was 94.6% for HPV DNA testing versus 55.4% for Pap testing ($P = 0.01$), with Pap testing having a small, but significant, advantage in terms of specificity (94.1% vs 96.8%; $P < 0.001$). On the liberal case definition, crude estimates (no correction for verification bias) were 82.8% versus 57.7% for sensitivity and 61.1% versus 80.9% for specificity for HPV DNA and Pap testing, respectively. With correction for verification bias on the liberal definition, sensitivity was reduced to 45.9% for

HPV DNA testing versus 43.4% for Pap testing, with a specificity of 94.2% versus 96.9%, respectively.

Negative predictive values were $> 99\%$ for both tests, regardless of case definition. Test performance was not affected by the sequence of the tests. Using the conservative definition, with correction for verification bias, sensitivity and specificity were 53.8% and 98.7%, respectively, when results were considered as Pap screening followed by HPV DNA triage, 53.8% and 99.1% for HPV DNA screening followed by Pap triage, and 100% and 92.5%, respectively, when results were considered as co-testing with the two tests. Based upon these three strategies, colposcopy referral rates would have been 1.6%, 1.1%, and 7.9%, respective-

ly, according to the study criteria.

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From the Oncologist's Perspective

Risk-based strategy for vaccination of the 19–26-year-old female population

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The most common sexually transmitted disease in the United States is caused by the human papillomavirus (HPV). Presently, Gardasil (HPV vaccine) is the only US Food and Drug Administration-approved vaccine for girls and women 9–26 years of age in the United States. Gardasil protects females from acquiring four specific types of HPV infection: HPV 16 and 18, which are associated with approximately 70% of cervical cancers, and HPV 6 and 11, which are associated with genital warts.

The Advisory Committee on Immunization Practices (ACIP) of the Centers for Disease Control and Prevention (CDC) recommends routine preventative HPV vaccination of 11- to

12-year-old girls, as well as subsequent vaccinations of girls and women 13 to 26 years of age who were not previously vaccinated or who have not completed the full series of three doses spread over 6 months.¹ As a result, several leading organizations, including the American College of Obstetrics and Gynecology, American Academy of Family Practitioners, American College of Physicians, and American Academy of Pediatrics, have all agreed with the ACIP recommendations and have provided similar recommendations for HPV vaccination. In addition, all of these organizations agreed that 9- to 10-year-old girls could be vaccinated, that females should be vaccinated regardless of previous HPV infection or abnormal Pap test results, and that Pap testing should be

continued after vaccination.¹ However, the American Cancer Society (ACS) promotes a more selective approach, with comprehensive vaccination recommended for all females younger than 19 years of age, but for women 19–26 years old, vaccination should be based on “an informed discussion between a woman and her healthcare provider about the likelihood of previous HPV exposure and potential benefit from vaccination.”

Challenges in this population

Despite these leading organizations' support of vaccinating females in the appropriate age group, in the 2 years since

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approval of the HPV vaccine, uptake in the 19- to 26-year-old population has lagged behind the uptake in the 11- to 18-year-old female group. So what are the challenges surrounding vaccination of this population of women? First, we need to examine the actual HPV vaccination rates in this group. Second, we need to identify the factors that contribute to low HPV vaccination rates. Third, we need to ask what we can do to improve vaccination rates in this population, focusing on whether the strategy of using risk factors to predict which groups of women should be targeted will contribute to improving this problem, as suggested by the ACS.

A recent CDC survey revealed low vaccination rates among adults.² Only 57% of adults 18–49 years of age received a tetanus vaccination in the past 10 years. Thirty-seven percent of adults 18–49 years of age at high risk for influenza received the annual influenza vaccine for the 2007 season. And only 10% of women 18–26 years of age have been vaccinated against HPV.

So what are the vaccination challenges in the US? Immunization has been the hallmark of pediatric preventive healthcare, and preventive healthcare visit patterns for infants and children have long been structured around routine vaccination schedules. What is known is that visits to primary care physicians decline substantially as adolescents grow and become teenagers, presenting fewer opportunities to vaccinate them.

Understanding the missed opportunities for childhood vaccination has been a focal point of most vaccination programs. However, less attention has been paid to understanding missed opportunities to vaccinate adults, even though the age-based adult vaccination schedule contains fewer vaccines and is much simpler to implement than pediatric vaccine schedules. Some of the more common obstacles that have been discussed for improving adult vaccination rates include discussing vaccines at patient visits, maintaining

and consistently using health-maintenance flow sheets, and scheduling preventive care visits.^{3–5}

Risk-based strategies

Risk-based vaccination strategies recently have been discussed as a means of targeting women at high risk for HPV vaccination if sufficient financing is unavailable for comprehensive immunization. Dempsey and colleagues⁶ evaluated the use of risk factors to determine a young woman's appropriateness for HPV vaccination among 3,276 sexually active women. They identified six risk factors previously associated with either HPV-related cervical disease or HPV infection that could be assessed during an outpatient clinical encounter. These risk factors included having:

- a sex partner more than 2 years older,
- more than three lifetime sex partners,
- a new sex partner in the past 12 months,
- use of illegal drugs in the past 12 months,
- sex while impaired by drinking alcohol, and
- never having been married.

The authors initially looked at the prevalence of HPV in sexually active women 18–26 years of age who had been included in the National Longitudinal Study of Adolescent Health. No women were positive to all four HPV types (6, 11, 16, 18) targeted by the HPV vaccine. Altogether, 0.4% of women were co-infected with both HPV 16 and 18. Looking specifically at the consequences of not vaccinating the estimated 2.5 million women who had more than three lifetime sexual partners, 12% would already be currently infected with one or more of the HPV types targeted by the vaccine. However, 88% would not be currently infected with HPV 6, 11, 16, or 18. Thus, an estimated 2.2 million women who could potentially benefit from receiving the HPV vaccine would not be vaccinated. The limita-

tions of a risk-based vaccination strategy are such that identification of individuals based upon either the presence or absence of risk factors for HPV infection therefore does not appear to be a viable strategy for HPV vaccination of young adult women. The ACIP also recognizes that "it is not possible for a clinician to assess the extent to which sexually active persons would benefit from vaccination, and the risk for HPV infection might continue as long as persons are sexually active."

So, the greatest opportunity for improvement of HPV vaccination rates in this population continues to be increasing public awareness of the availability of the vaccine, promoting discussion about the administration of vaccines at each patient clinical encounter, and continued evaluation of adult vaccination schedules to ensure that women receive protection from acquiring HPV infections.

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HPV testing in cervical cancer screening: a pathologist's perspective

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The goal of cervical cancer screening is to triage only women with a high risk of developing invasive cervical carcinoma and high-grade precursors to colposcopy. With our evolving understanding that persistent infection with high-risk human papilloma-virus (HPV) types is necessary for the development of cervical carcinoma and its precursors, HPV testing for these types has become an integral adjunct to Papanicolaou (Pap) smears.

Pap smears detect the cytologic changes associated with HPV infection but are relatively insensitive and have an inherent false-negative rate. High-risk HPV testing detects current infection; however, it is persistent infection and the consequent dysplastic cytologic changes that are most clinically relevant. At present, cervical cancer screening combines both cytology and high-risk HPV testing, and there are two US Food and Drug Administration (FDA)-approved tests for this purpose: digene (QIAGEN; Germantown, Md), which detects 13 high-risk HPV types, and Cervista HPV HR and HPV 16/18 (Hologic, Inc; Bedford, Mass), which detects 14 high-risk HPV types (HPV HR) and genotypes positive cases (HPV 16/18).

Competing technologies

Digene uses proprietary Hybrid Capture 2 technology, a nucleic acid hybridization assay with signal amplification using microplate chemiluminescence for the qualitative de-

tection of 13 high-risk HPV types in cervical specimens, including types 16 and 18, which are associated with approximately 70% of cervical cancers in the United States. Positivity indicates infection with one or more high-risk HPV types, but not the specific type. Digene is used for reflex testing of women with atypical squamous cells of undetermined significance (ASCUS) on Pap screening, as well as for routine testing as an adjunct to Pap tests in women over 30 years of age.¹ FDA approval for these purposes was reached in 1999 and 2003, respectively, after validation in the ASCUS-LSIL Triage Study.^{2,3}

Cervista HPV HR and Cervista HPV 16/18 tests were FDA approved in March 2009. They use Invader chemistry technology, a signal amplification method for detection of specific nucleic acid sequences using two isothermal reactions. Cervista HR qualitatively detects 14 high-risk HPV types and is approved for the same purposes as digene. Cervista HPV 16/18 uses the same technology and specifically detects HPV 16 and 18.⁴ Validation was performed in the Cervista HPV HR multicenter clinical trial.⁵ There is increasing evidence that in women older than 30 years of age with negative cytology, positivity for HPV 16 or 18 confers a significantly greater 10-year risk for CIN 3 (cervical squamous intraepithelial neoplasia 3) than the risk of CIN 3 in similarly aged women who are positive for any other high-risk HPV type;

FDA approval of this HPV genotyping test paves the way for incorporation of these data into management.⁶

Cervical cancer screening guidelines

Since 2006, the American Society for Colposcopy and Cervical Pathology (ASCCP) guidelines for interpretation and follow-up of abnormal cervical cancer screening tests have included high-risk HPV testing and cytologic results. Women with ASCUS on a Pap smear who are high-risk HPV positive are triaged to colposcopy, whereas those who are HPV negative should return in 1 year for a repeat Pap test. Women over age 30 with negative cytology and high-risk HPV positivity should return for Pap and HPV testing in 1 year, whereas those who are negative for both HPV and cytology may wait 3 years before being screened again.⁷ However, the recent FDA approval of Cervista has resulted in amended 2009 guidelines for women over age 30 with negative cytology. The ASCCP guidelines now recommend that women aged 30 and older with negative cytology and high-risk HPV positivity undergo HPV 16/18 genotyping. Women who are positive for HPV type 16 or 18 should be triaged to colposcopy, whereas those who are negative for both HPV types should be rescreened in 1 year.⁸

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Open issues

Integration of high-risk HPV testing and results into management algorithms in cervical cancer screening is evolving and raises many issues for pathologists. First, although there are currently two FDA-approved tests, there are other tests under FDA review (eg, Roche Diagnostics' AMPLICOR HPV Test and LINEAR ARRAY HPV Genotyping Test⁹). Interestingly, FDA approval is not necessary for HPV or many other laboratory tests, and there are laboratories that use non-FDA-approved platforms. High-risk HPV testing demonstrates the tradeoff between sensitivity and specificity; though the desire is to detect women with persistent high-risk HPV infections that predispose to the development of cervical carcinoma and high-grade precursors, it is critical that the test not be so sensitive as to unnecessarily triage women to colposcopy.¹⁰

A second consideration is that the recent change in ASCCP guidelines may be a challenge to those laboratories that do not currently perform HPV genotyping. Finally, the crowning debate is whether high-risk HPV testing will replace cytology. There are proponents of both sides of this issue, but for now, both the Pap smear and high-risk HPV testing remain important parts of cervical cancer screening.

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