The Potential Utility of HPV Genotyping in Screening and Clinical Management

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Key Words
Cytology, cervical intraepithelial neoplasia, CIN, cervical precancer, cervical cancer, human papillomavirus, HPV genotyping

Abstract
Detection of specific human papillomavirus (HPV) genotypes, or HPV genotyping, may be useful for differentiating between those women who are carcinogenic HPV-positive at lower and higher risk for cervical precancer and cancer. Considerable evidence already exists that the absolute risk for cervical precancer and cancer varies considerably among specific HPV genotypes, and that detection of HPV-16 and -18 may have clinical usefulness, especially among women who tested positive for carcinogenic HPV and have negative cytology. Detection of persistent carcinogenic HPV is strongly associated with cervical precancer and cancer and strongly predicts its development, and might be used to monitor the outcomes of HPV infections. However, several practical considerations must be addressed before HPV genotyping can be used in screening and clinical management. (UNCCN 2008;6:83–95)

Papanicolaou (Pap) tests, and more recently liquid-based cytology, have been successfully implemented for secondary prevention of cervical cancer. Epidemiologic data and ecologic correlations of incidence/mortality trends of cervical cancer with cytology screening activities in populations have provided evidence of significant reductions in cervical cancer rates.1 In the United States, rates of cervical cancer have declined dramatically, with only 11,150 cases and 3670 deaths projected in 2007.2

Given the success of the Pap test and other cytologic methods, one may question the introduction of alternative screening modalities. However, although successful, cytology-based programs have several limitations,1 including poor sensitivity for precancerous lesions4,5 and poor reproducibility.6 As a consequence of frequently applied, inefficient screening, the effective cytology-based program in the United States is costly. In 1992, the estimated annual cost of the cervical cancer prevention program based on Pap tests was $6 billion.7 Undoubtedly, these costs have risen in the past 15 years. Much interest has been shown in improving the efficiency of cervical cancer screening programs while maintaining the overall effectiveness.

Natural History of Human Papillomavirus and Carcinogenic Human Papillomavirus Testing

Laboratory and epidemiologic data have clearly shown that cervical infections from approximately 15 cancer-associated (carcinogenic) human papillomaviruses (HPV) genotypes cause virtually all cervical cancer8,9 and its immediate precursor lesion,10 cervical intraepithelial neoplasia grade III (CIN III) or carcinoma in situ (cervical precancer). In most populations, HPV infection is commonplace and virtually all sexually active people have been exposed to HPV at least once in their lifetime. Most HPV infections are benign and transient, clearing within 1 to 2 years. Unresolved carcinogenic HPV infections confer an increasing risk for development of cervical precancer, which if not detected and treated, can become invasive.
The stages of cervical carcinogenesis can now be summarized as 1) HPV acquisition, 2) HPV persistence (vs. clearance), 3) precancer (CIN III) development, and 4) invasion.11

In the context of this cervical carcinogenesis model, the success of well-established cytology programs in detecting cervical precancer and treatable cancer was and is partially attributable to repeated screening of women during the slow progression from incident HPV infection to precancer (typically 2–10 years) and from precancer to cancer (typically ≥ 10–15 years).

Based on knowledge of the central role for persistent carcinogenic HPV in cervical carcinogenesis, testing has been introduced to improve the efficiency and maximize the sensitivity of cervical cancer screening; one test for carcinogenic HPV is already FDA-approved (Hybrid Capture 2 [hc2], Digene Corporation, Gaithersburg, Maryland) and other tests will soon be widely available. Convincing evidence now shows that carcinogenic HPV DNA testing is cost-effective and sensitive for detecting precancerous lesions in women with equivocal cytology;12–17 is more sensitive (and negative test provides greater reassurance), although less specific for primary cervical cancer screening;14,18–22 can be added to the follow-up of women postcolposcopy when precancer is not found,23 and can guide assessment of cure posttreatment.24–26 The greater reproducibility of current tests for carcinogenic HPV27–28 is an added advantage over cytology.4 In the United States, HPV testing is commonly used to triage equivocal cytology. HPV testing with cytology is also approved for primary screening of women aged 30 years and older who are past the peak of self-limited infections and whose positive predictive value (PPV) for cervical precancer and cancer is higher than that of younger women.28 Women aged 30 years and older who test negative for carcinogenic HPV and are cytologic negative are at an extremely low risk for incident CIN III and cancer over 10 years, and therefore the screening intervals in these women can be extended to 3 years in the United States to make cotesting cost-effective. The International Agency for Research on Cancer has endorsed the use of carcinogenic HPV testing alone as an option in primary cervical cancer screening. Several clinical trials are evaluating carcinogenic HPV testing in primary screening and should provide supporting evidence for this recommendation.

### Rationale for Genotyping

Despite its promise, carcinogenic HPV testing has an important limitation: poor PPV. That is, carcinogenic HPV testing cannot distinguish between those who test positive and have clinically irrelevant, transient infections from those who test positive and have cancer, precancer, or persistent carcinogenic HPV infections. HPV genotype-specific detection, or HPV genotyping, may be useful for differentiating lower or higher risk in women who test carcinogenic HPV-positive, because risk for cervical precancer and cancer (≥ CIN III) varies among carcinogenic HPV genotypes and genotype-specific persistence is strongly linked to presence or development of ≥ CIN III.

Considerable evidence already shows that the absolute risk of ≥ CIN III varies considerably between specific HPV genotypes10 and that detection of HPV-16 and -18 may have clinical usefulness, especially among carcinogenic women who have positive HPV tests and negative cytology.11 HPV-16 is the most carcinogenic HPV genotype and accounts for approximately 55% of all cervical cancer worldwide; HPV-18 is the second most carcinogenic HPV genotype and accounts for another 15% to 20% of cervical cancer. Moreover, for not understood reasons, HPV-18 is as or more important than HPV-16 as the cause of cervical adenocarcinoma.13 Although a rarer histologic genotype than squamous cell carcinoma, adenocarcinoma has become increasingly more common in well-screened populations, such as the United States and Europe,13 and can account for up to 20% of all cervical cancer cases in those populations.

Given that HPV-16 and -18 infections account for 70% of all cervical cancer and only 30% are attributable to the other approximately 13 carcinogenic HPV genotypes, HPV-16 and -18 should warrant special consideration. Evidence suggests that their separate detection may be clinically useful for identifying women at greater risk for ≥ CIN III. In one study, detection of HPV-16 and -18, even among cytologically normal women, conveyed a greater 10-year risk for ≥ CIN III than atypical squamous cells of undetermined significance (ASCUS) or low-grade squamous intraepithelial lesion (LSIL) cytology (Table 1). Women who test positive for HPV-16 or -18 and are cytologically negative had an approximately 20% risk for ≥ CIN III over 10 years, compared with a risk of approximately 1% to 2% for
all other carcinogenic HPV genotypes combined (Figure 1). That is, HPV-16 and -18 infections denoted a much greater absolute risk (PPV) for CIN III than the absolute risk for other genotypes in aggregate. However, the latter still account for a sizeable fraction (30%) of disease despite being less carcinogenic.

Other studies have confirmed the importance of HPV-16 detection in screening populations. However, findings have been inconsistent on the next important type for detection: HPV-18 versus HPV-31 or -33. Why these differences exist is unclear. One possible explanation is population differences in the relative importance in HPV genotypes after HPV-16. However, a recent meta-analysis has shown that HPV-18 is the second most common genotype in invasive cancer on all continents and the most important genotype in adenosquamous cancer/

### Table 1 Comparison of the 10-year Cumulative Incidence Rate of CIN Grade III and Cancer (≥ CIN III) Through Cytologic Interpretation and HPV Risk Group*

<table>
<thead>
<tr>
<th>HPV Status</th>
<th>Negative</th>
<th>ASC</th>
<th>LSIL</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>HPV-16+</td>
<td>20.7 (8.6–32.8)</td>
<td>7.7 (0.0–22.2)</td>
<td>30.0 (1.6–58.4)</td>
<td>20.1 (9.7–30.6)</td>
</tr>
<tr>
<td>HPV-18+</td>
<td>17.7 (0.0–36.0)</td>
<td>0.0</td>
<td>0.0</td>
<td>15.4 (0.0–31.7)</td>
</tr>
<tr>
<td>Carcinogenic HPV+ (excluding HPV-16 and -18)</td>
<td>1.5 (0.3–2.7)</td>
<td>6.4 (0.0–13.4)</td>
<td>4.0 (0.0–11.7)</td>
<td>1.8 (0.6–3.0)</td>
</tr>
<tr>
<td>Carcinogenic HPV−</td>
<td>0.5 (0.3–0.7)</td>
<td>3.3 (0.1–6.6)</td>
<td>9.1 (0.0–26.1)</td>
<td>0.5 (0.3–0.8)</td>
</tr>
<tr>
<td>Total</td>
<td>0.8 (0.5–1.0)</td>
<td>4.2 (1.3–7.1)</td>
<td>11.1 (1.5–20.7)</td>
<td></td>
</tr>
</tbody>
</table>

*HPV-16+ > HPV-18+ > carcinogenic HPV+ (excluding HPV-16 and -18) > carcinogenic HPV−.

Abbreviations: ASC, atypical squamous cells; CI, confidence interval; CIN, cervical intraepithelial neoplasia; CIR, cumulative incidence rate; HPV, human papillomavirus; LSIL, low-grade squamous intraepithelial lesion.

adenocarcinoma, which is more easily missed in cytology-based screening programs. A more likely explanation is the underrepresentation of HPV-18–induced precancerous lesions. For unknown reasons, HPV-18 may produce more occult lesions, and this phenotype is perhaps related to the unique relationship of HPV-18 with adenosquamous cancer/adenocarcinoma. As corroborative evidence, prevalently detected HPV-18 infections are much less likely to be accompanied by high-grade squamous intraepithelial lesion (HSIL) cytology than HPV-16, even though the genotypes are similarly likely to have any concomitant nonnormal cytologic interpretation, as shown in Figure 2. HPV-18 infections can persist for long periods before cancer is diagnosed, with minimal clinical indications of abnormality (Table 2; Rolando Herrero, MD, PhD, and Ana Cecilia Rodriguez, MD, Proyecto Epidemiologico Guanacaste, unpublished data). Given the obvious importance as the second most carcinogenic HPV genotype and the tendency to cause occult precancerous lesions, a clear rationale exists for monitoring HPV-18 in the population. Evidence does not seem to support the usefulness of separate one-time detection of other carcinogenic HPV individually for clinical management decisions.

A potentially more powerful use of HPV genotyping assays could be the monitoring of genotype-specific HPV infections. Detection of persistent carcinogenic HPV is strongly associated with and strongly predicts the development of precancer, and might be used to monitor the outcomes of HPV infections. Carcinogenic HPV genotypes, particularly HPV-16, and older age are associated with an increased likelihood of persistent HPV infection.

The likelihood of persistence of HPV infections seems to depend on the previous duration. That is, the longer HPV persistence of a given genotype, the less likely the infection will clear (Figure 3) and the greater the risk for precancer. Long-term persistence is a strong but imperfect predictor of carcinogenicity, because some noncarcinogenic genotypes show long persistence (e.g., HPV-61). Prevalently detected infections persist longer in older than younger women, possibly because they are more likely to represent infections already of long duration. The observed median time to clearance of HPV infections detected during screening studies ranges from 6 to 18 months. No accepted definition of clinically important persistence exists, but follow-up strategies targeting abnormalities lasting more than approximately 1 year begin to distinguish infections and associated lesions that pose greater risk from those posing lower risk. The small percentage (~10%) of carcinogenic infections persisting several years (2–5) is strongly linked to a high absolute risk for precancer diagnosis and over longer periods linked to cancer.

Epidemiologic studies have shown that risk for cervical precancer and cancer correlate strongly with patterns of HPV genotypes (i.e., changes in HPV genotypes detected over time modulate risk). For example, women who are reclassified into lower- or higher-risk groups of HPV (HPV-16 > carcinogenic HPV excluding HPV-16 > noncarcinogenic HPV > HPV-negative) have lower or higher risk for ≥CIN III, respectively. Persistence of a given carcinogenic HPV genotype poses a greater risk for cervical precancerous lesions than testing positive for different carcinogenic HPV genotypes on repeat visits.
HPV Genotyping in Screening and Management

Table 2 Case History*

<table>
<thead>
<tr>
<th>Months Since Enrollment</th>
<th>Pap Smear CR</th>
<th>JHU</th>
<th>LBC</th>
<th>Cervigram Result</th>
<th>HPV Types</th>
<th>Colposcopy</th>
<th>Histology</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>Reactive</td>
<td>Normal</td>
<td>N/A</td>
<td>Normal</td>
<td>18/51</td>
<td></td>
<td></td>
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<tr>
<td>12</td>
<td>Reactive</td>
<td>Normal</td>
<td>Reactive</td>
<td>Normal</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>24</td>
<td>Reactive</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>Normal</td>
<td>Reactive</td>
<td>Normal</td>
<td>Normal</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>49</td>
<td>Inadequate</td>
<td>Normal</td>
<td>Inadequate</td>
<td>Normal</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>56</td>
<td>Reactive</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>18</td>
<td></td>
<td>Cancer</td>
</tr>
<tr>
<td>61</td>
<td>Reactive</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>18</td>
<td>Inadequate</td>
<td></td>
</tr>
<tr>
<td>72</td>
<td>Reactive</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>98</td>
<td>Reactive</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>108</td>
<td>Reactive</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>109</td>
<td>CIN1</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>116</td>
<td>CIN3</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>18</td>
<td></td>
<td></td>
</tr>
<tr>
<td>117</td>
<td>Reactive</td>
<td>Normal</td>
<td>Normal</td>
<td>Normal</td>
<td>18</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Patient is a 28-year-old woman diagnosed with cervical cancer participating in the Guanacaste Project, a 7-year prospective study of HPV infection and cervical neoplasia among 10,000 women in Guanacaste, Costa Rica. Note the false-negative polymerase chain reaction result for HPV-18 at month 37, emphasizing the importance of reliability in viral monitoring.

Abbreviations: CIN, cervical intraepithelial neoplasia; CR, Costa Rica; HPV, human papillomavirus; JHU, Johns Hopkins University; LBC, liquid-based cytology.

Source: Unpublished data, courtesy of Rolando Herrero, MD, PhD, and Ana Cecilia Rodriguez, MD, Proyecto Epidemiologico Guanacaste.

Risk Assessment

Before discussing how HPV genotyping may be applied to cervical cancer screening, a framework for using it should be introduced: risk stratification. The concept of risk stratification (distinguishing the few women at risk from the many who are not at risk) is the principle underlying any screening test. In general, the risk for ≥CIN III within the subsequent 2 to 3 years could be assigned a management option, independent of how the risk was measured. A more extensive discussion of risk stratification in cervical cancer screening has been presented elsewhere and therefore is only discussed briefly as it relates to HPV genotyping.

HPV genotyping may provide a more powerful risk stratification than previously achievable with cytology and carcinogenic HPV testing alone. Testing negative for carcinogenic HPV provides reassurance, but testing positive has a poor PPV for disease (i.e., most women who test positive for carcinogenic HPV do not have ≥CIN III). A useful strategy would be to further stratify women who test positive for carcinogenic HPV into those who might require immediate colposcopy for safety and those who could be followed up more intensively than by routine screening alone. Those who test positive for HPV-16 or -18 have an elevated risk compared with those who test positive for other carcinogenic HPV genotypes and might warrant
colposcopy. Women who test positive for carcinogenic HPV for whom HPV-16 and -18 infections have been ruled out, might warrant watchful waiting until they show evidence of elevated risk (e.g., viral persistence).

The absolute risk for ≥ CIN III in women who test positive for HPV-16 (and perhaps those with evidence of long-term viral persistence) in any population is very high, and in some circumstances could warrant treatment in the absence of a confirmatory biopsy diagnosis, given the less-than-perfect sensitivity of colposcopy to detect precancer. In the ASCUS-LSIL Triage Study, women who tested positive for HPV-16 at baseline had a risk of 30% to 40% for ≥ CIN III and a risk of approximately 50% for ≥ CIN II within 2 years. In combination with enrollment cytology of HSIL, these risks were 61% and 81% (Philip Castle, PhD, MPH, unpublished data; Figure 4). With colposcopic impression of high-grade or worse, these risks increase to 81% and 97% (Castle, unpublished data). With the current limitations of 70% sensitivity for detecting precancerous lesions through colposcopy, cases will arise in which the risk profile exceeds the ability of the colposcopist to find small precancerous lesions. Treating women at very high-risk may be more cost-effective and less invasive than requiring them to have 2 or more clinical visits, including an intensive follow-up with multiple biopsies to find the precancerous lesion that is likely already present but invades, and to then treat them.

Applications
Screening
The most useful application of HPV genotyping is in primary screening, although its introduction could take many forms, such as alone, with cytology, and with carcinogenic HPV testing and cytology. HPV testing may soon be an accepted option (provided that an internal control exists for determining specimen adequacy) for primary screening, after large screening trials are concluded confirming that HPV testing is more sensitive than cytology. A validated and reliable HPV genotyping test could be used to identify the population at risk, such as those who test positive for carcinogenic HPV, and the subpopulation at the highest risk for disease warranting immediate clinical attention: those who test positive for HPV-16 or -18, or are persistently infected with any carcinogenic HPV genotype. A possible, simplified scenario based on clinical response according to approximate risk for each condition is presented in Figure 5.

Carcinogenic HPV testing is restricted to being an adjunct to cytology for general screening of women aged 30 years and older, because a positive carcinogenic HPV test has a greater PPV in these slightly older women, who on a population average will have less transient and more clinically relevant HPV. In other words, age is a surrogate marker of risk for HPV persistence or worse. Because HPV genotyping could potentially better discriminate between more- and less-risky HPV through actually measuring HPV persistence, these assays could be applied to slightly younger women. Although the ideal age is uncertain, introducing HPV genotyping in the United States and Europe for viral monitoring around 25 years of age when the risk for precancer in the general population begins to rise would seem plausible.

HPV-16– and -18–vaccinated populations will show a reduced burden of related infections and cervical precancer and cancer. However, as a direct consequence, the performance of current screening tests will be negatively impacted because the 2 most important biomarkers of risk, HPV-16 and -18, will largely be absent in approximately 10 years when these populations...
will require screening for the approximately 30% of precancer and cancer that the vaccines do not protect against. Specifically, PPV will decline significantly, as projected by epidemiologic studies, so that a simple HPV test will have limited ability to risk stratify; that is, the meaning of a positive screening test will be more ambiguous. Monitoring persistence of these weaker carcinogens could potentially improve the PPV of screening in populations vaccinated with HPV-16 and -18 through better identifying those at risk.

**Triage of Mildly Abnormal Cytology**

A more controversial use of HPV genotyping is among women who have carcinogenic HPV-positive ASCUS or LSIL, which have nearly identical risks for precancer and should be considered the same condition. Women who test positive for HPV-16 in these populations have very high risks for ≥ CIN II and ≥ CIN III over a couple of years. The risk in this population might warrant treatment under certain circumstances. Even if colposcopy is performed and no lesion is observed, women who are positive for HPV-16 with ASCUS or LSIL might benefit from careful surveillance until they show evidence of viral clearance.

In contrast, women who test negative for HPV-16 and positive for carcinogenic HPV with ASCUS or LSIL have a 10% or less 2-year risk for ≥ CIN III. This risk is very similar to that attributed to ASCUS unqualified by HPV testing, which may or may not have led to immediate colposcopy. The risk in these women is comparable to the risk for recurrence in those who have undergone excision for a precancerous lesion, and these women are followed-up closely but not immediately referred for colposcopy unless their screening test is positive. Applying similar arguments based on a risk model for cervical cancer prevention, monitoring these women rather than referring them for colposcopy and possible treatment of equivocal disease may be acceptable, especially for those of reproductive ages for whom overtreatment has important negative consequences on pregnancy outcomes.
Posttreatment Monitoring for Recurrence

Treatment of cervical precancerous lesions through excision is highly effective, with a 90% to 95% cure efficacy. Recurrence in 5% to 10% of women suggests genotype-specific persistence, which strongly implies failed treatment. Unsurprisingly, data show that genotype-specific persistence strongly predicts short-term recurrence. Conversely, the absence of the causal genotype implies successful treatment and a low risk for recurrence; women who test negative for the causal type are likely to return safely to routine screening (appropriate to their risk of cervical precancer and cancer), but this clinical algorithm must be verified.

Misuse of HPV Genotyping

Strategies for using HPV genotyping have the caveat that these tests have a tremendous potential for misuse and frank abuse. Troubling evidence already shows widespread misuse of carcinogenic HPV testing, some of which is clearly profit-driven, and introducing HPV genotyping into clinical practice could exacerbate the situation. Conducting more tests without changing clinical practice only increases the costs of screening without providing greater programmatic efficiency or patient benefit. For example, current guidelines recommend that women who have negative cytology and a positive carcinogenic HPV test return for screening in 6 to 12 months. Experts have suggested that when HPV genotyping with a validated clinical test becomes available, women who test positive for HPV-16 or -18 might benefit from immediate colposcopy, whereas those who test negative only must handle the infections and possible HPV genotypes once a test is available. Any screening of these women using these tests is unwarranted, unethical, and should be discontinued, because they derive no benefit and many will test positive for HPV, causing unnecessary anxiety and fear.

Practical Considerations

Several practical categories of consideration for conducting HPV genotyping assays to detect women at elevated risk for cervical precancer and cancer warrant discussion: 1) clinical performance, 2) reliability, 3) cost, 4) provider and patient acceptability, 5) practical clinical algorithms, 6) women lost to follow-up, and 7) user-friendliness.

Clinical Performance

Detection of each HPV type should be at least 90% to 95% sensitive for detecting related ≥CIN III, with perhaps a more rigorous requirement for detecting HPV-16 and -18 because related precancers are more likely to invade. However, the PPV for ≥CIN III, as a corollary of specificity, will decrease significantly if the analytic sensitivity for detecting individual HPV genotypes is too great.

Reliability

These assays will be used to monitor approximately 15 carcinogenic HPV genotype-specific infections...
over 1 to 2 years to establish genotype-specific persistence and risk for ≥ CIN III. This is a high bar for reliability. Most current HPV genotyping assays rely on polymerase chain reaction (PCR) amplification of a 60- to 450-base pair region of the L1 gene using consensus primers (i.e., targeting conserved regions of the gene). The limitation of this approach is possible competition for the primers by coinfection with a transient infection of any genotype, carcinogenic or not, resulting in a false-negative test for the clinically relevant HPV genotype. Clinicians must be able to rely on a positive or negative result to equate with persistence or clearance, respectively, for HPV genotyping to monitor persistence to be clinically useful. Skip patterns or intermittent positivity (e.g., positive/negative/positive) for any carcinogenic HPV genotype will create greater ambiguity rather than clarity risk. Because of the potential errors in monitoring approximately 15 HPV genotypes, relying on tests that detect carcinogenic HPV in aggregate and that separate detection of the most carcinogenic HPV genotypes (i.e., HPV-16 and -18) may be more robust. Repeat positive tests for carcinogenic HPV may serve as a useful and reliably measured surrogate for detection of genotype-specific HPV persistence.

**Cost**

The objective of using HPV genotyping (or any assay) is to risk stratify and match the appropriate clinical care to that risk. If the results of HPV genotyping are used indiscriminately (i.e., no differentiation between women at greater and lesser risk), HPV genotyping will increase only the costs of screening and not patient benefit.

**Clinician and Patient Acceptability**

Related to cost, use of HPV genotyping implies both clinician and patient acceptability. Specifically, clinicians must be willing to recommend and follow through with the appropriate management based on the risk linked to the test outcome. For example, HPV-16 detection may warrant colposcopy even if cytology is negative, and patients (and clinicians) must accept a positive HPV test result and be willing to wait for the follow-up test result in approximately 1 year to see if the infection clears or persists before making a clinical decision. Doctors seeing patients for the first time will be faced with an unknown or inaccurately reported clinical history.

Medicolegal pressures inspire a tendency to overreact to the first positive result.

**Practical Clinical Algorithms**

Clinicians, who have many clinical responsibilities, will not want to routinely obtain data on approximately 15 HPV genotypes. Most will not be familiar with each genotype and associated risks, leading to confusion. Simple algorithms must be developed to provide clinicians with the clinically relevant information (e.g., HPV-16/18–positive and persistent carcinogenic HPV) linked to management recommended by a professional clinical society (e.g., American Society for Colposcopy and Cervical Pathology and/or American College of Obstetricians and Gynecologists).

**Lost to Follow-Up**

Detection of genotype-specific HPV persistence requires follow-up. In addition to irregularities of routine screening or poor compliance with recommendations, women may move or switch medical programs or primary clinicians. In many cases, data are not linked. Therefore, given an HPV-positive test result, clinicians will be uncertain how long the patient has harbored the infection. Cytology may be a particularly useful adjunct (Figure 5) when history is unknown.

**User Friendliness**

Clinical laboratories are faced with a wide array of clinical tests. To make HPV genotyping practical for clinical laboratory use, assays must be fully automated, including specimen preparation, and provide rapid, high through-put results. Otherwise, laboratories may not adopt the technology or the costs of performing the test will make it prohibitively expensive.

First and foremost, any HPV genotyping assay must be validated, with demonstrated reliable clinical performance in detecting ≥ CIN III. The focus should be on detecting CIN III rather than CIN II, because CIN II is a heterogeneous mixture of CIN I, which is merely a productive viral infection and can be caused by both carcinogenic and noncarcinogenic HPV genotypes, and precancer (CIN III). As a benchmark, the detection of carcinogenic HPV genotypes in aggregate should achieve between 90% to 95% sensitivity for ≥ CIN III found within a screening interval; note, however, that because of the limitations in the sensitivity of colposcopy, not all prevalent ≥ CIN III will be found immediately. Specificity of 85% or better
and κ values of 0.7 or better are needed. Although these standards are for carcinogenic HPV detection in aggregate, they provide a benchmark for the clinical detection of each individual genotype (i.e., the detection of any HPV genotype should be at least 90%–95% sensitive for related ≥ CIN III, and so on). However, given the greater potential for HPV-16– and -18–related precancer to invade, requiring a slightly higher bar of clinical sensitivity for detecting these types may be reasonable.

However, the analytic sensitivity requirements for an HPV genotyping assay remain less clear and may depend on how it is applied to screening. For detecting disease within 1 to 2 years, the optimal analytic sensitivity seems to be approximately what is achieved with current tests, which are already 90% to 95% sensitive for the detection of ≥ CIN III. Further increases in analytic sensitivity will only result in increased detection of HPV genotypes in women without precancer or cancer (i.e., little gain in clinical sensitivity), and significant reductions in specificity and PPV. Ideally, positive cut-points for each genotype should be considered individually and based on a receiver-operator curve analysis. However, when an HPV genotyping assay is used as a secondary test for women whose carcinogenic HPV-positive status is identified through a screening test for carcinogenic HPV in aggregate, a lower limit in analytic sensitivity has been set by the primary screening test (i.e., reflex testing with a more analytically sensitive HPV genotyping after an hc2–positive result will be restricted to the analytic sensitivity of hc2).

Regarding their use as a stand-alone screening test for detecting the most carcinogenic HPV genotypes and monitoring viral persistence as a risk factor for progression, increases in analytic sensitivity will undoubtedly hurt the PPV for detecting all types and, most importantly, HPV-16 and -18. However, monitoring HPV viral patterns may benefit from added analytic sensitivity because of the need for great fidelity in detecting each genotype for multiple measurements before diagnosis. If greater analytic sensitivity can be achieved without increases in test noise (false-positives), increased analytic sensitivity would increase the signal-to-noise ratio and therefore improve the reliability/reproducibility of HPV detection (e.g., current assays are more reliable in populations of women with non-normal cytology because the high viral load translates into better signal-to-noise ratios). Slightly increased analytic sensitivity could buffer against the variability caused by specimen collection and possible dropout in detecting the important genotype, a characteristic of PCR amplification assays that rely on L1 consensus primer PCR because of competition for primers in multigenotype infections. The optimal positive cut-points for using HPV genotyping to monitor the natural history of infections have not been established, and different parameters must be balanced carefully to achieve the desired reliable performance.

Future Research

How the risk for ≥ CIN III is modulated by increasing duration of infection is unknown. As a corollary, how long persistent carcinogenic HPV infections can be monitored safely before the associated risk is high enough to warrant treatment, despite the absence of diagnostic confirmation of precancer, is also unknown. Almost certainly this relationship is genotype-specific, with the most carcinogenic HPV genotypes on average more rapidly transitioning to precancer than the less-carcinogenic HPV genotypes. Infections from HPV-16 are particularly likely to make that transition very early, so that HPV persistence becomes synonymous with the presence of precancer, although the CIN III lesion may not be detected until it is large enough to be visualized in a colposcopic examination, sometimes years later.

Finally, advancements in colposcopy are necessary to fully appreciate the benefits of HPV genotyping in identifying women at greatest risk for precancer while less aggressively managing those at lower risk. Without these improvements, the distinctions made with HPV genotyping will be nullified by the limitations in colposcopy, and clinicians, faced with the medicolegal pressures to unrealistically prevent all cancer, will lose confidence in its use.

Conclusions

Based on the fundamental role of HPV persistence in the development of cervical precancer and cancer, a strong rationale exists for detecting and monitoring HPV infections in screening and clinical management to predict risk. HPV genotyping has the clinical potential to improve cervical cancer screening through identifying women at very high risk for cervical precancer and cancer among those at risk (i.e., those with...
a positive carcinogenic HPV test). However, several practical concerns must be addressed, including the development of well-validated, user-friendly tests with reliable clinical performance, before HPV genotyping can improve the accuracy of screening and focus clinical management on those at greatest risk. Of equal concern is whether these assays will be used judiciously to improve patient management and care.

References


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