Status of HPV testing
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Summary: The status of HPV testing of cervical samples is up in the air. There is certainly no consensus of its utility. There are many conflicting claims in the literature as to the utility of testing for HPV. "For HPV testing to be a cost effective option, sensitivity for detection of CIN 2/3 must consistently provide a reassurance that allows triage on the basis of an HPV test result without the requirement for a repeat office visit and Pap smear" (1).

The Hybrid Capture II test is reported to be 50-100 times more sensitive than the first version (1, 2), which was neither sensitive nor specific (3, 4). The ability to detect High-grade and cancer in women "at risk" (whatever that means) with the Hybrid Capture II test is reported to be greater than 90% sensitive. However, the gain in sensitivity is offset by even lower specificity than for Hybrid Capture I (2). The positive predictive values ranged from 10 to 35% for ASCUS (2), which means that most positives were false positives.

Therefore, using the Hybrid Capture II test to triage ASCUS cases will result in a large number of women undergoing unnecessary biopsies, which triaging was supposed to prevent. The argument used to get around this problem is that the more-important concern is the false negative rate. But that's not a problem with the current protocol where all ASCUS cases are evaluated extensively over the course of a couple of years to catch the true positives.

Analysis

HPV typing would be of value in the assessment of cervical biopsies if it were able to: 1. discriminate between reactive and neoplastic lesions; 2. discriminate between viral infection and intraepithelial neoplasia; 3. distinguish the morphologically defined grades of CIN; or 4. predict those lesions which will progress to higher grades or invasive carcinoma. Initial molecular studies suggested that high-risk HPV infection was restricted to high-grade CIN and invasive cervical carcinoma. However, it has become clear that a significant proportion of low-grade lesions (both 'pure' HPV infection and CIN 1), most of which regress, contain these viral types. Although low-risk HPV types are only very rarely found in high-grade CIN, they are also only found in a minority of low-grade lesions in the absence of condylomatous architecture. This indicates that neither demonstration of HPV nor determination of HPV type is of value in the assessment of CIN. *Herrington and Wells 1997* (5)

It has been suggested that HPV testing has a role in both primary cervical screening and as an adjunct to current cytology-based screening, particularly where low-grade cytological abnormalities are identified (5). However, given the evidence that HPV sequences are frequently present in smears from patients with low-grade cervical cytological abnormalities but without high-grade cervical disease (6), and that prospective analysis of such patients does not show them to be at greater risk of lesion progression (7), the predictive value of HPV DNA detection is significantly limited (5).

Several HPV DNA detection methods have been described during the last decade, each of which allows the detection of a wide spectrum of HPV types, but none has fulfilled all expectations (8). There are now over 100 types of HPV (9). Virtually every paper on testing HPV in cervical samples justifies the approach in the first paragraph by declaring that HPV is carcinogenic. HPV is said to cause most cervical cancer. If that were true, it would make a great deal of sense to test for the presence of HPV. However, HPV does not cause cervical cancer, or any other cancer for that matter. But I will leave that discussion to the end.

Setting aside for now the question of HPV causing cervical cancer, there are still a great many other issues that bear on whether or not to test for the virus. First, HPV infection is ubiquitous in adult populations of the USA and Europe (10, 11), which amounts to hundreds of millions of people. Yet there are only 12,000 cervical cancers in the USA each year. If HPV causes cervical cancer it is a very weak carcinogen. The shear ubiquity of HPV infection renders testing for the virus of little clinical utility (12).

An important study on sexually active teen-aged girls has shown that the viral types may change with the passage of time (13). Also, an elaborate study of viral typing in precancerous lesions of the uterine cervix documented that in approximately 33% of the high-grade lesions, the viral types have not been identified (14). The overall rate of documented HPV infection in a group of female college students was 46%, reaching 100% for women in the age group 22-23 years who had 10 or more sexual partners. Furthermore, 20% of virgins were also carriers of the virus (15). And, there is no evidence that a woman bearer of a high-risk virus will necessarily develop a neoplastic lesion.

Notwithstanding the arguments above, there is a great deal of literature evaluating the utility of HPV testing of cervical specimens. A report entitled "Relevance of human papillomavirus screening in management of cervical intraepithelial neoplasia" found that more than 40% of women with confirmed cervical intraepithelial neoplasia grades 2 and 3 would not have had colposcopy and biopsy performed had HPV DNA testing alone been used for triage in patients with low grade lesions on Pap smears (3). On top of that, there was an overall false positive rate of 65%. The authors concluded that "human papillomavirus screening does not appear to be of value to identify women with abnormal Papanicolaou smears who can be safely followed up with cytologic study alone." According to Table 5 in a paper by Solomon et al., (16) the Hybrid Capture 2 HPV test has positive predictive value (PPV) equal to 10% for CIN3 and higher and PPV=20% for

CIN2 and higher. In other words, 80-90% of Hybrid Capture 2 positive results are false positives in a large sampling of women.

A recent study that compared aneuploidy and HPV stated that, "the validity of aneuploidy to detect HSIL [high grade] in our results was much higher than that of HPV typing; positive predictive value, 97% versus 35% and negative predictive value, 76% versus 68%. …Besides the DNA grading of squamous intraepithelial lesions, we also see DNA cytometry of Pap smears as a diagnostic tool for difficult lesions, for example, for the interpretation of atypical endocervical glandular cells, atypical squamous cells of undetermined significance (ASCUS) and postirradiation smears" (17).

The natural history of papillomavirus infection has raised additional doubts as to the utility of HPV testing of cervical samples (18, 19). Of the 246 women who had an abnormal cervical smear, 40% tested negative for HPV and another 33% tested positive for the first time only at the same visit as the abnormal smear (18). "Thus for only 21% of women was a positive HPV test predictive of abnormal cytology at a subsequent visit, and the cumulative risk at 3 years was 33%…[And] persistence of HPV infection did not confer the largest risk of an abnormal smear" (19).

The development of neoplasia "in a woman who has a positive HPV test may be due more to the play of chance than most investigators have assumed" (19).

"A positive HPV test especially in young women, rarely represents disease that could, if unrecognized, progress to cervical cancer…[T]he low sensitivity of the corresponding cervical smear is largely spurious. So the use of HPV testing risks the overtreatment of more non-progressive disease than does the cervical smear" (19).

The intrinsic risk of overtreatment from being HPV-positive is compounded by the 80-90% false positive rate of Hybrid Capture 2 (16).

Prior to Thursday's New England Journal of Medicine article (which I have not had a chance to examine), the most optimistic report on the utility of the Hybrid Capture II test comes from **Israel**. Using a select group of women with repeated ASCUS cytology results (69% prevalence of CIN), the authors reported a sensitivity for detecting high-grade lesions of 86% and a specificity of 97% (20). With the exception of the NEJM paper that I am looking forward to reading, the Israelis are all alone in reporting such excellent results.

In contrast to the Israelis and the McGill paper in the upcoming NEJM paper, a **British** study of a cross-section of women from London with mild or borderline conditions were tested for HPV using the Hybrid Capture II test (21). The sensitivity of the test to detect high-grade lesions was 90% but the false positive rate was 42%. The authors concluded that, due to the low sensitivity and specificity "further improvements in the technique are needed before it can be used as a triage strategy."

In an attempt to find utility for the Hybrid Capture II test, some investigators are moving away from triaging ASCUS into even more specific niches. For example, Lin et al. evaluated the use of the Hybrid Capture II test in women 50 years of age and older (22). They report a sensitivity for 100% for detecting high-grade lesions, a false negative rate of 0%, and a false positive rate of 33%. The authors restricted their investigation to older women because "cervical HPV infection in younger women is usually transient; that is one explanation for the less-than-ideal positive predictive value of HPV testing in studies involving younger women." The authors added that, "a mediocre positive predictive value [or high false positive rate] may result in a relatively large number of women undergoing unnecessary biopsies."

A **French** study confirmed the low sensitivity (71%) and quite low positive predictive value (17%) of the Hybrid Capture I test (23). For that reason, they evaluated the second generation test. The authors showed that HPV infection peaked in women between the ages of 21-30 years. The study population was not random but from women at high risk for sexually transmitted diseases. The reported sensitivity for detecting high-grade lesions was 100%, but the positive predictive value was again quite low at 13%. Over 85% of positive test results were false positives. In the authors' words: "Thus even if it is less specific, HC-II [Hybrid Capture II] represents a more sensitive test than classic cytology for the detection of high-grade cervical lesions."

The CDC compared the Hybrid Capture II test with the PCR gold-standard for HPV and concluded that the "complex probe cocktails may result in false-positive results, and such results should be cautiously interpreted" (9).

To summarize, even if it turns out that testing for HPV may provide some meaningful information, in order for the test to be optimally cost effective, a positive threshold will need to be set at different levels for women at different ages, since it is clear that increased sensitivity results in decreased specificity, particularly for younger women (1, 24). Furthermore, since so-called oncogenic types of HPV are commonly found in women with low-grade, equivocal, and normal diagnoses, the criteria for referring women to colposcopy based on the Hybrid Capture II test will depend on the prevalence of HPV in a population, which depends largely, in turn, on age-specific societal sexual practices for each locale (25).

Because HPV infection per se cannot be cured, testing for viral types will only increase costs of screening without tangible benefits to society but with high levels of anxiety generated in women testing positive but disease-free (26). Finally, Herrington and colleagues have thoroughly studied the pros and cons of HPV testing and have concluded that, "Current evidence does not support the introduction of routine HPV typing in histology or cytology" (5).

Does HPV really cause cervical cancer?

No!

Herpes simplex virus (HSV) was postulated in the 1970s to be the cause of cervical cancer based on epidemiological correlations with HSV DNA (27). In the 1980s, another virus, human papilloma virus (HPV), was postulated, again based on epidemiological correlations, to be the causative factor in cervical and anogenital cancers (27-29). The epidemiological correlations are the only evidence offered as proof that these viruses cause cancer, in spite of the fact that epidemiology cannot prove causation—only disprove it. There is no functional evidence that either of these viruses can cause cancer. Moreover, the HPV- and HSV-cancer hypotheses offer no explanation for the absence of a reciprocal venereal male carcinoma (30).

Both HSV and HPV are transmitted sexually and other ways. Both are ubiquitous in the USA and Europe (27, 29). While HSV typically kills infected cells (27), HPV naturally replicates nonlytically, forming polyclonal warts with unintegrated viral DNA (31). Different sets and amounts of HSV or HPV DNA are integrated into the cell DNA of different carcinomas but the DNA is defective and is either poorly expressed in some cancers or not expressed at all in others (27, 29, 32). But no set of viral genes is consistently present or expressed in human cervical cancers, or even in cells from the same tumor (30).

Therefore, the "hit-and-run" mechanism of viral carcinogenesis was proposed. It holds that neither the complete virus, nor even a part of it, needs to be present in the tumor (33). Obviously, this is unfalsifiable, but also an unprovable hypothesis since there are cervical cancers without a trace of either HSV or HPV (30).

As mentioned above, HPV does not replicate in cancer cells and there are no HPV-specific histological or physiological markers that set HPV DNA-positive apart from negative carcinomas (29). There is also no virus-specific integration site in HPV DNA-positive cancers (29), indicating that no specific cellular gene is activated, or that a tumor suppressor gene is inactivated by integration of viral DNA. In addition, HPV DNA-positive tumors are clonal and carry clonal chromosomal abnormalities, just like virus-negative tumors (29, 34, 35).

Detecting inactive and defective HPV DNA in carcinomas is a fossil record of a prior infection that is irrelevant to carcinogenesis.

Thus, detecting inactive and defective viral DNA from past infections in non-tumorigenic cells with commercial hybridization test or with PCR seems worthless as a predictor of rare carcinomas appearing decades later, in view of the "ubiquity" (29) of these viruses in women and the lack of evidence that cervical cancer occurs in women with HPV more often than those without (30). In fact, the testing for HPV may be harmful, considering the anxiety a positive result induces in believers of the virus-cancer hypothesis.

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