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Note

[1]*Editor's note:* SEER is a set of geographically defined, population-based central tumor registries in the United States, operated by local nonprofit organizations under contract to the National Cancer Institute (NCI). Each registry annually submits its cases to the NCI on a computer tape. These computer tapes are then edited by the NCI and made available for analysis.

New Epidemiology of Human Papillomavirus Infection and Cervical Neoplasia

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The prospective study of cervical intraepithelial neoplasia (CIN) reported by Ho et al. (*1*) in this issue of the Journal illustrates how the epidemiologic study of cervical neoplasia has evolved during the past 10-15 years. The epidemiologic interview studies of the 1960s and 1970s correctly identified the venereal nature of cervical neoplasia, but they could not pinpoint a specific etiologic agent. Now the primary exposure measurements are DNA tests for the types of human papillomaviruses (HPVs) proven during the past decade to cause most cases of cervical neoplasia (*2*).

The new epidemiologic model incorporating a central role for HPV infection has spawned many testable hypotheses regarding the natural history of HPV and carcinogenic cofactors. Thus, molecular epidemiology groups, similar to the group of Ho et al. can focus intensely on clarifying particular stages in the natural history of HPV-induced neoplasia. In this issue of the Journal, Ho et al. have demonstrated how persistent detection of HPV DNA (especially high levels of DNA) is linked to persistent diagnosis of CIN when both are measured repeatedly over time.

To put the contribution of Ho et al. into context, the presumed stages of cervical carcinogenesis must be briefly summarized. As presently understood, the first stage of cervical carcinogenesis is transmission to the cervical epithelium of an oncogenic type of HPV (of which there are about 15 types). Usually, transmission is via sexual intercourse (*3*). Although necessary for nearly all cases of cervical neoplasia, most initial cervical infections with an oncogenic HPV type do not lead to a diagnosed cytologic abnormality, perhaps because cytologic screening is irregularly performed and relatively insensitive to minute foci of infection. When infection does result in a diagnosis of cervical neoplasia, it is usually a low-grade lesion (CIN 1, including koilocytotic atypia) (*4*), which is microscopically apparent within 1 or 2 years of infection. The early cytologic changes following HPV infection, and the infection itself, usually regress spontaneously over months to a few years because of a host response thought primarily to be mediated by the cellular arm of the immune system (*5,6*) (Tsukui T, Hildesheim A, Schiffman MH, Lucci J, Contois D, Lawler P, et al.: manuscript submitted for publication). While regression occurs in most cases, eventual progression of lesions to CIN 3 is seen in a small minority of cases, with the risk of progression increasing the longer the lower grade lesions persist (*2*). If left untreated, women with CIN 3 lesions commonly develop invasive cervical cancer over the course of years (*7*). Whether chronic or acute, the vast majority of all grades of cervical neoplasia have detectable HPV DNA (*8-10*). HPV-negative cervical neoplasia is a rare though non-negligible event (≤10% of all cases).

In their careful prospective study, Ho et al. (*1*) focused on the determinants of persistence and progression versus regression of CIN in 100 women with lesions originally diagnosed as CIN 2. CIN 2 is a troublesome, borderline category to epidemiologists and clinicians hoping to dichotomize CIN as either low grade (the mild and usually transient cytologic effect of HPV) or high grade (the fixed cancer precursor requiring immediate treatment). Ho et al. demonstrated the biologic and/or diagnostic heterogeneity of CIN 2 when they observed one third of their 100 presumed cases to "regress" immediately to normalcy. Given such data and the common observation that some CIN 2 lesions contain HPV types not found alone in cancers (*8,10*), it may be that many cases of CIN 2 are low-risk lesions, while only severe CIN 2 lesions (perhaps marked by aneuploidy) should be conceptually joined with CIN 3 in the high-grade category (*11*).

By following their remaining 70 patients with repeated examinations up to 15 months, Ho et al. attempted to find determinants of later regression versus persistence and progression of CIN. As mentioned above, the key host factor influencing the natural history of CIN is probably cell-mediated immunity, which Ho et al. did not assess. Instead, their study examined viral factors as well as behavioral factors assessed by questionnaire. The repeated HPV test measurements were especially complete and proved informative. This group has promoted studying HPV and CIN at multiple "levels" of detection, starting at the most common, lowest level infections detectable only by polymerase chain reaction-based DNA detection (*12*). Accord-

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ingly, the investigators repeatedly measured HPV infection at the molecular level (amplified and unamplified DNA testing for more than 20 types of HPV), microscopic level (cytologic smears and biopsy specimens), and clinical level (colposcopic visualization). The resultant dataset was a complicated but informative set of repeated, correlated measurements, far from the classic two-by-two table (exposure versus disease) at the conceptual heart of traditional epidemiology.

Thanks to their effort, Ho et al. could demonstrate that the persistence of CIN mirrored the persistence of HPV infection measured at the molecular (HPV DNA) level. Of note, persistent high levels of HPV detection (positive repeatedly by Southern blot) were most predictive of persistent CIN. Thus, the natural history of CIN moved synchronously with the ebb and flow of HPV DNA detectability (viral load). This finding is reasonable, given that the abnormal cells diagnosed microscopically as CIN are the factories of HPV virion production.

The finding of repeated correlations of HPV and CIN over time confirms ample and similar cross-sectional (point prevalence) data (*13,14*). Moreover, the study results strengthen the evidence that persistence (versus transience) of infection is the crucial variable explaining how HPV can be simultaneously one of the most common sexually transmitted infections and also the central cause of cervical neoplasia. It should be acknowledged that this group (*15*) first suggested the crucial role of viral persistence in HPV natural history by analogy to hepatitis B carcinogenicity.

Although it is now understood that most HPV infections "go away by themselves," the details of transience and persistence are largely unknown. These details will prove crucial in developing screening strategies that include HPV DNA testing. In particular, the state of HPV preceding the development of CIN 3 and cervical cancer remains an important, unanswered screening question, because these are the lesions that absolutely must be detected with high sensitivity. Suppose for a minute that all cases of CIN 3 and cancer were derived from previous milder CIN. If, as observed by Ho et al., high levels of HPV DNA are associated with persistence and progression of CIN, then it might be feasible to add HPV DNA testing for high levels of virus to Pap smears. Limiting the classification of HPV positive to high-level detection might improve the sensitivity and negative predictive value of screening for incipient high-grade CIN (i.e., to catch false-negative Pap smear results), without severely limiting the positive predictive value. Perhaps it will be important to restrict this screening strategy to older women, in whom persistent (rather than acute and transient) infections are likely to predominate.

This potentially important strategy should be evaluated in earnest. However, on the basis of our unpublished incidence data from follow-up of more than 17 000 women for up to 5 years, some cases of CIN 3 may arise from an alternative causal pathway. We are observing about 5%-10% of the first abnormal cytologic diagnoses following HPV infection to be CIN 2 or even CIN 3, rather than CIN 1. Thus, women occasionally develop CIN 3 as the first cytologic evidence of CIN as apparently incident disease rather than progression of CIN 1 or 2 (*16*). Although this route to CIN 3 is probably a relatively minor causal pathway, our preliminary data suggest that HPV DNA levels preceding incident CIN 2-3 may be low, not high as observed by Ho et al. for progressive CIN. If this puzzling difference proves to be true, then an amplified HPV test similar to the polymerase chain reaction may be required to guarantee detection of all women with incipient high-grade lesions. If an amplified test is required, the positive predictive value of screening is likely to decline. The key question will be by how much.

The methodologic complications raised by epidemiologists' efforts to study multistage cervical carcinogenesis are worth considering. The most challenging issues involve the intersection of cross-sectional misclassification and time. Apparent progressions, persistence, or regressions from one stage in pathogenesis to the next unavoidably comprise a mixture of true changes and misclassifications. The misclassifications make it difficult to determine temporal relationships. For example, an HPV test of a CIN 1 lesion may show high levels of an oncogenic HPV type. If the patient's disease progresses quickly to CIN 3, it is unclear whether the HPV test at base line predicted a true subsequent transition or signaled that the CIN 3 was already present but missed at base line. This difficulty is unavoidable because all of the levels of measuring HPV or CIN are prone to error; thus, no reference standard exists of either infection or disease.

With this caveat, Ho et al. appeared to show that HPV infection mirrors the current state of CIN more strongly than the future state of CIN. In other words, the HPV measurements at one interval better predicted CIN at the same interval than at the following interval. Similarly, among cytologically normal women, we have observed that HPV DNA detection predicts the coexistence of CIN with a cross-sectional odds ratio that is an order of magnitude greater than the relative risk for future incidence of CIN. Clearly, HPV infection cannot be reduced to a simple, all-purpose epidemiologic variable. Because of issues of transience and persistence, as well as high rates of new infection, prospective and prevalent risk estimates are not interchangeable.

Another area of complexity in the epidemiology of multistage carcinogenesis is discriminating lesions from organs. The cervix is topographically a ring, not a point. Therefore, similar to the skin or the bowel mucosa, the cervical epithelium may have several discrete lesions with separate natural histories (*17*). Ho et al. justifiably ignored this issue to avoid paralyzing complexity, but the issue eventually must be addressed. Does the appearance of a CIN 1 lesion at the posterior lip of the cervix truly represent persistence (versus regression and reinfection) if the original lesion, now disappeared, was at the anterior lip? How often do CIN 3 lesions emerge directly from CIN 1 lesions rather than adjacent to them? In addition to painstaking clonality studies, ongoing natural history investigations are now emphasizing the visual level of measuring HPV by using cervicography (*18*) or recorded colposcopy to track individual lesions and to better define their separate natural histories.

To the epidemiologic methodologist, studies of multistage cervical carcinogenesis require a rethinking of traditional approaches. The statistical analysis of repeated measurements depends on the "black box" of modeling time-dependent covariates. No reader can easily challenge the findings of Ho et al. because the raw data are never presented by interval. In fact,

we must trust the summary, with its underlying statistical assumptions, because the underlying data are too complex to digest. Ho et al. made several simplifying analytic choices regarding HPV detection and typing, as well as the grading of disease severity. For example, under their analytic model, if a woman were HPV positive, then negative, then positive, and then positive, she would be included in both the nonpersistent and persistent groups, although the single negative test measurement could easily be false. (The infection may have persisted throughout.) This type of unavoidable misclassification probably has decreased the strength of the associations they report, but by how much is unclear. Statistical methods available for the analysis of repeated measurements (*19,20*) are just recently becoming popular for studying the transient and reversible states of HPV infection and CIN 1. Most of us still need to learn to use and understand the methods better.

None of these concerns overwhelm the enthusiasm of those of us working on the increasingly specialized epidemiology of HPV and cervical neoplasia. We are contributing to the rapid construction of a new causal paradigm. Working on the epidemiology of cervical neoplasia is now reminiscent of completing a puzzle whose broad outlines are already visible; placing any remaining piece provides a satisfying confirmation of the correctness of the overall design.

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