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The Use of HPV Seroepidemiology to Inform Vaccine Policy

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Introduction

In this issue, Dunne et al. (1) report on the seroprevalence of four types of human papillomavirus (HPV) in a study of 499 men from Tucson, Arizona and Tampa, Florida. They observed increasing seropositivity with age for HPV6/11, the major causes of genital warts and respiratory papillomatosis, and for HPV16 and HPV 18, the major causes of anogenital cancers and some oropharyngeal cancers. Men who smoked were more likely to be seropositive to at least one of the types, as were those who had a current sexual partner in the past 3 months. Interestingly, in contrast to findings from studies in women, the association of seropositivity with lifetime sexual partners was weak. Overall, seroprevalence to HPV 16 was 12.1%, to HPV 6/11 combined was 9.7%, and to HPV 18 was 5.4%; only 1% of men were positive on all three type-specific assays.

HPV6, 11, 16, and 18 are the four types prevented by the currently licensed HPV prophylactic vaccine (Merck's Gardasil). The results suggest that benefit from quadrivalent vaccination would decrease with age due to increasing previous exposure resulting in immunity to re-infection. Viewed uncritically, the data also would suggest that considerable benefit in men might remain well into adulthood because seroprevalence did not increase substantially until the 35+ year old age group.

Any tool to guide the planning of vaccine programs is welcome, and this study represented a fruitful collaboration of excellent epidemiology and serology. As the authors state, vaccine recommendations in men are both topical and important. Policies must weigh the high cost of male vaccination programs that would cost billions of dollars against possible benefits that include prevention of genital warts and a fraction of anogenital and oral cancers in men and their sexual partners. Adding male vaccination to female vaccination programs would also help prevent, to a still unknown amount, the great burden of cervical cancer/precancer in women.

It is important for readers to put the paper by Dunne et al. in context, with realization of the complexity of HPV serology compared with other viral infections in which viremia leads reliably to antibody titers that predict subsequent immunity. The proper and exact use of cross-sectional HPV serosurveys in formulation of vaccination policy is made difficult by 1) diverse assays that are not yet fully developed or comparable and 2) our incomplete

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understanding of what different serology assays are measuring with regard to HPV exposure and immunity, especially in men.

Use of serology to measure cumulative HPV exposure, immunity to subsequent infection, and risk of subsequent neoplasia

As mentioned by Dunne et al., HPV serology is helpful to the degree that it correctly measures two related phenomena, represented in Table 1: 1) HPV natural history, specifically cumulative exposure in the past and especially the subset of infections that lead to 2) subsequent immunity to infections that could cause future disease. At present, we presume that immunity results from exposure (i.e., that virtually all individuals are initially susceptible). It seems that HPV immunity in natural history is type-specific, although the very high titers of antibody following vaccination might provide some cross-protection against closely related HPV types. However, the fractions of exposures that lead to seroconversion, the percentages of infected individuals who lose antibody positivity over time, and the relationship of seropositivity to immunity are all inadequately understood. Therefore, the relative percentages in the rows and columns of Table 1 have not been defined for any given serologic assay.

The research needs are clear, but difficult to achieve. HPV DNA testing is the reference standard of present infection, but clearance (reversion to DNA negativity) occurs in the majority of infections, often quite quickly within a few months (2). Measuring DNA one time does not tell us about cumulative exposures that might have occurred, including the important subset that might have left the individual immune to re-infection. To estimate how well serologic assays summarize cumulative exposure, we would need type-specific serologic measurements at the end of a long, longitudinal series of HPV DNA genotyping tests. Such longitudinal comparisons in men are not yet available (and such analyses are incomplete in women).

Even more important, we would like to use serology to measure future susceptibility versus immunity to subsequent HPV infections that increase the risk of important disease (genital warts or cancer). To know how well serologic assays predict susceptibility/immunity, we would need to follow men who are seropositive or seronegative due to natural exposure (not vaccination that produces very high titers of antibody), observing the subsequent incidence of infection and disease. Again, adequate studies in men have not been reported (and even the data in women are very scant).

Biologic complexity that makes serology even more difficult to interpret

If HPV infections were restricted to only one tissue, we would still need to relate seropositivity to cumulative exposure and future immunity. But the situation is even more complicated, because HPV of given types can infect multiple tissues and HPV natural history appears to vary by tissue. For example, HPV DNA prevalence tends to decline with age at the best-studied tissue (the female cervix), but prevalence might not decline as much by age at the anus or penis. The reasons are unknown.

A serologic assay is by its nature not tissue-specific, and necessarily assesses immune recognition of HPV infection anywhere in the body. To understand thoroughly what seropositivity means in men, we would need to compare serology to past DNA and future DNA/pathology assessed repeatedly, with fairly short sampling intervals, in all infected tissues (penis, anus, oral cavity, etc.). HPV 6/11, 16, and 18 commonly infect anogenital and oral skin and mucosa. Longitudinal studies of these and other HPV types in men are just beginning. Observations from women might or might not apply to men. For example, approximately one half of women who test positive for cervical HPV DNA seroconvert to that type, with viral persistence leading to increased seroconversion (3;4). It is not known whether the same percentages apply to men. Dunne et al. found that seropositivity rose markedly around age 35 and was not strongly related to lifetime numbers of partners, suggesting lower probability of durable seroconversion given infection.

Susceptibility to infection and risk of subsequent cancer are not strictly related throughout the body. The risk of cancer from HPV is greatest in transformation zones where different kinds of epithelium meet, like the squamocolumnar junction of the anus (5). The oropharynx also has a transformation zone. Other infected tissues without transformation zones like the mouth and penis, even if commonly infected, uncommonly develop invasive cancers caused by HPV.

As an additional complexity, HPV serology measures antibodies, but clearance of infections that do occur and immunity to subsequent infection might relate to cell-mediated immunity, which antibody assays might not measure well. Finally, there is evidence that serologic assays vary by HPV type. Dunne et al. measured the most important HPV types in men. HPV18 seropositivity was lower than HPV16 seropositivity, in line with relative prevalences of these types as measured by DNA assays. It is interesting that many authors report an anomalous finding in women of similar prevalences of HPV18 and HPV16, despite HPV16 being more prevalent based on DNA (4). Perhaps, in women, the serologic response to HPV18 might not be the same as for HPV16. This might reflect biologic or test differences.

Kinds of HPV serology assays

Various HPV serology assays with different properties are currently available (Table 2), and it is not possible to compare seroprevalence across assays. They differ quantitatively, i.e., by throughput and detection range, but also qualitatively, i.e., whether they detect an antibody response directed against multiple epitopes (indicative of previous exposure) or whether they specifically detect neutralizing antibodies that might confer immunity (protection against subsequent infection). Thus, the choice of serology assay determines which scientific questions can be addressed.

The most commonly used kind of assay detects a wide range of antibodies directed against HPV, not strictly neutralizing antibodies. For example, the assay used in the study by Dunne et al. is based on virus-like particles (VLPs) bound to ELISA plates by capture antibodies targeting neutralizing epitopes (VLP capture ELISA,) (6;7). This type of assay ensures that only intact VLPs serve as epitopes and that antibodies directed at neutralizing epitopes can

be measured. However, the assay can detect antibodies directed against all conformational epitopes presented in correctly folded VLPs.

Clearly, a major challenge of developing HPV serology assays is the complex structure of HPV particles. Most relevant epitopes are conformational, disqualifying simple peptide-based assays and assays based on denatured recombinant protein. Minor changes of viral particles may alter the native conformational structure and eliminate important epitopes (8). Although recombinant L1 can be expressed at high quantities and purified easily, one must validate that unassembled recombinant L1 displays all important epitopes (9).

High-quality VLPs based on self-assembling HPV L1 proteins are not trivial to generate, but they are useful because they are very similar to native HPV in terms of target antigens. VLPs can be coupled directly on untreated ELISA plates (10), on heparin-coated plates to reduce the background (11), or using capture antibodies as done by Dunne et al. (6). An L1-Luminex assay uses GST to couple recombinant L1 to Luminex beads (12). Similar to above-described assays, the L1-Luminex assay detects antibodies directed against HPV-L1, including protective and non-protective antibodies. The advantage is the ability to rapidly and inexpensively test many different HPV types.

Several strategies have been developed to specifically measure protective antibody responses against HPV. Hemagglutination of mouse erythrocytes mediated through correctly assembled VLPs can be inhibited by antibodies directed against neutralizing epitopes (13). However, not all neutralizing antibodies prevent hemagglutination, limiting the sensitivity of this approach. The development of recombinant VLPs with a serine alkaline phosphatase reporter system (SEAP) has improved the measurement of protective antibodies (14). Since the assay is based on an *in vitro* infection model, theoretically all neutralizing antibodies can be measured in the SEAP ELISA. However, the method is very laborious, not suited for high throughput, and sensitivity for studies of natural history where antibody titers are very low may be limited. Recently, a competitive assay to measure protective antibody responses has been developed (15;16). The competitive Luminex based Immunoassay (cLIA) measures the presence of antibodies directed against the HPV16 V5 epitope that compete against labeled V5 antibodies. Analogous neutralizing antibodies for HPV6, 11, and 18 can be detected simultaneously in the same specimen. This assay might have great promise. Data from the unvaccinated women based on this assay in a vaccine trial have demonstrated that HPV16 seropositive, DNA negative women had a very low rate of subsequent HPV16-related CIN2+ (17). The sensitivity of the assay does not appear to be high, however. While seropositivity predicts immunity, seronegativity might not imply susceptibility in many instances.

In summary, serology assays measuring a wide range of antibodies directed against HPV are designed to indicate previous exposure, while neutralizing serology assays should indicate who might be protected from subsequent infections. At present, the results do not accurately reflect what the assays are designed to measure. Also, although we can assume that the results obtained with the different assay types are correlated, we currently lack sufficient comparison data between HPV serology assays. We have only very limited HPV serology data generated with modern assays in natural history studies. Translating findings from

vaccinated women to natural infections may be limited by quantitative and qualitative differences in antibody responses.

Conclusion

With this background, the authors' conclusion can be considered along with its nuances: *In summary, this study found that many sexually active men, especially with increasing age, have evidence of exposure to at least one vaccine-type HPV infection. Few men had antibodies to all 4 vaccine types. This information could be useful when considering HPV vaccine use in men*.

If we knew what fraction of cumulative HPV infections were captured as seropositive, we could proceed as follows for policy considerations: We could correct for false negativity (i.e., by doubling or tripling or whatever multiplier is correct) the observed seroprevalence to estimate true cumulative population exposure. Then, we could subtract that percentage from 100% to estimate true percentage unexposed. All unexposed individuals could be presumed to be immunologically naïve and susceptible to future infection, although a sizable fraction might not be subsequently exposed and another sizable fraction would never develop meaningful disease even if subsequently infected. However, without more methods work in the context of longitudinal cohorts, we are not sure how to extrapolate from current assays to move from seropositivity estimates to prediction of cumulative exposure or future susceptibility. Inaccurate estimations could mislead as much as they inform; we urge caution in using cross-sectional serosurveys to inform vaccine policy at this time.

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Table 1

Possible interpretations of HPV serology results, as measurements of cumulative exposure and future immunity/susceptibility to individual HPV types

Serology Result	True Cumulative Exposure	True Future Immunity/Susceptibility	Meaning of Result Relative to Cumulative Exposure
Positive	Unexposed	Susceptible	False positive serology
Positive	Unexposed	Immune	Not likely to occur
Positive	Exposed	Susceptible	True positive serology, exposed but susceptible
Positive	Exposed	Immune	True positive serology, exposed and immune
Negative	Unexposed	Susceptible	True negative serology
Negative	Unexposed	Immune	Not likely to occur
Negative	Exposed	Susceptible	False negative serology, exposed but susceptible
Negative	Exposed	Immune	False negative serology, exposed and immune

Table 2

Kinds of HPV serology assays currently available

Intended measure	Name	Platform	Antigen	Type coverage	Coupling	Remark	Throughput, volume requirement	Dynamic range	References
Exposure	VLP capture ELISA	ELISA	VLP	Dependent on VLP and neutralizing AB availability	Neutralizing antibody (e.g. HPV16-V5)	Capture antibody for conformational epitopes	High throughput, low sample volume	Medium (about 2 log)	(6;7)
Exposure	VLP ELISA	ELISA	VLP	Dependent on VLP availability	Direct/ Heparin	Purified VLPs important, Different modifications	High throughput, low sample volume	Medium (about 2 log)	(10;11)
Exposure	VLP Luminex	Luminex	VLP	Dependent on VLP availability	Covalent binding to Luminex beads		High throughput, multiplexing, low sample volume required	Wide (3–4 log)	NA
Exposure	Multiplex serology	Luminex	Recombinant L1	Wide coverage	GST-fusion	Bacterial background	High throughput, multiplexing, low sample volume	Wide (3–4 log)	(12)
Protection	Hemagglutinin inhibition assay	Microtiter plates	VLP	Dependent on VLP availability	NA	Indirect measure of neutralization, only a subset of neutralizing ABs can be detected	Low throughput	Low, low sensitivity	(13)
Protection	Secreted alkaline phosphatase neutralization assay (SEAP)	Microtiter plates	SEAP-VLPs	Dependent on SEAP-VLP availability	293TT cell infection	Very laborious	Low throughput, high sample volume	Low, low sensitivity	(14)
Protection	Competitive Luminex-based immunoassay (cLIA)	Luminex	VLP	Dependent on VLP and neutralizing AB availability	Covalent binding to Luminex beads		High throughput, multiplexing, high sample volume	Wide (3–4 log)	(15;16)

VLP=virus like particle; AB=antibody; NA=not available; SEAP=Secreted alkaline phosphatase