Since the first diagnostic HIV test in 1985, HIV testing has become easier, more sensitive, more accessible, and less invasive. Unfortunately, national data indicate that a large percentage of new human immunodeficiency virus (HIV) diagnoses are made in the late stages of disease. In the United States, 40% of patients with newly diagnosed HIV infection develop AIDS within one year of testing. Furthermore, it has been estimated that one-fourth of the people living with HIV infection in the United States do not know they are infected and, thus, miss the opportunity to receive life-saving antiretroviral therapy. That lack of awareness of infection is critical from the public-health perspective as it is estimated that over 50% of new sexually transmitted HIV infections are due to people unaware of their HIV infection; and evidence strongly suggests that once individuals are made aware of their infection, they reduce their risk behavior and decrease the probability of transmitting infection. Therefore, HIV testing provides an opportunity for decreasing the continued incidence of HIV infection and for providing life-saving therapy to newly diagnosed patients.

To decrease the number of people unaware of their HIV infection, in 2006, the Centers for Disease Control and Prevention (CDC) expanded its HIV-testing recommendations. The new CDC recommendations advocate voluntary “opt-out” HIV screening in healthcare settings for all adults instead of just screening traditionally “high-risk” patients. The recommendations also suggest eliminating requirements for written consent for HIV testing, annual re-testing for persons with known risk factors, and third-trimester screening for women who test negative for HIV early in pregnancy. This article will review the current HIV-testing types, with a focus on the role of the various testing modalities in both the public-health and clinical arenas. An additional emphasis will be placed on newer testing modalities and strategies, specifically rapid testing and nucleic-acid amplification testing (NAAT).
HIV virology and natural history

Our increasing understanding of HIV-transmission kinetics and the clinical course of the infection has changed the strategies used for HIV testing. HIV is a retrovirus that consists of an envelope and a viral core. The viral envelope is taken from the membrane of a human cell during viral budding and carries Env — a complex viral protein. Env consists of a cap (made from glycoprotein gp120) and a stem (made of gp41). Within the envelope, the viral capsid is made of thousands of copies of another viral protein, p24. These three proteins are highly antigenic and are used in many diagnostic tests. The capsid surrounds two single strands of HIV RNA, each of which has a copy of the virus’s nine genes.

A transient immunoglobulin M (IgM) antibody response against the capsid or envelope proteins is usually the first to appear and is followed by a long-lasting immunoglobulin G (IgG) response. The appearance of IgG antibodies against the core (p24) and envelope (gp160, 120, 41) proteins are then followed by antibodies against HIV viral enzymes. The development of detectable antibodies against different HIV proteins is called HIV seroconversion and marks the end of what is called the “window period.” Acute HIV infection is a term usually referring to the dynamic period between HIV infection and seroconversion.

Acute HIV infection is followed by a period of clinical latency that may last up to 10 years or longer. During this time, viral replication remains active and causes persistent activation of the immune system and subsequent progressive decline in CD4 T-cell lymphocyte counts. Both viral and host factors will determine the rate of decline in the CD4 T-cell count which, ultimately, results in loss of immune function and increased susceptibility to opportunistic infections.

The new CDC recommendations advocate voluntary “opt-out” HIV screening in healthcare settings for all adults instead of just screening traditionally “high-risk” patients.

HIV is among the most genetically variable of human pathogens. In the United States, HIV-1 accounts for the great majority of cases. A similar but different virus, HIV-2, is a common cause of AIDS in several areas of western Africa but is rare in the United States. In 1998, the CDC reported only 79 cases of HIV-2 infection in the United States.4 A great variety of HIV-1 strains have been identified and further divided into groups. HIV-1 group M strains are responsible for the most HIV infections worldwide, but the global distribution of HIV is complex and dynamic, and the prevalence of different strains could change rapidly. HIV-1 group O is endemic in Cameroon; and while it is uncommon in America, a case of HIV-1 group O was first reported in the United States in 1996.6

Host and viral markers of infection are used for diagnosis and can change during the natural history of HIV infection.5 Within the first few days of HIV infection, the virus starts an active replication, enabling the detection of viral RNA. Viral antigens (proteins) become detectable soon after. The protein used most widely in HIV diagnosis is the capsid protein p24, which appears usually within the first two to three weeks after acute infection. Its presence in the serum is transient, and its disappearance coincides with the development of both humoral and cellular responses to infection.

Table 1. Selected enzyme immunoassays

<table>
<thead>
<tr>
<th>Generation</th>
<th>Mechanism</th>
<th>Approximate window period</th>
<th>Name</th>
<th>Sample</th>
<th>Target molecule</th>
<th>Manufacturer</th>
<th>FDA cleared</th>
</tr>
</thead>
<tbody>
<tr>
<td>First and second generation</td>
<td>Viral lysate used to bind patient HIV Ab. Detects IgG antibody to HIV viral proteins. Second generations are the same as first generation, but use purified Ag or recombinant virus.</td>
<td>4 to 12 weeks</td>
<td>Vironostika HIV-1 Microelisa</td>
<td>Serum/Plasma/ Blood spot/Gingival fluids</td>
<td>Viral Lysate</td>
<td>bioMerieux Inc.</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Genetic Systems HIV-1 EIA</td>
<td>Plasma/Blood spot</td>
<td>Viral Lysate and E-coli recombinant antigen</td>
<td>Bio-Rad Laboratories</td>
<td>Yes</td>
</tr>
<tr>
<td>Third generation</td>
<td>Same mechanism as first and second generation, but adds IgM detection, which decreases the window period</td>
<td>3 to 4 weeks</td>
<td>HIVAB HIV-1/HIV-2 (rDNA)</td>
<td>Serum/Plasma</td>
<td>Recombinant HIV-1 env and gag HIV-2 env proteins</td>
<td>Abbott Laboratories</td>
<td>Yes</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Genetic Systems HIV-1/HIV-2 PLUS O EIA</td>
<td>Serum/Plasma</td>
<td>Purified gp160, p24, and peptides representing regions of gp41 from HIV-1 group O and gp36 from HIV-2</td>
<td>Bio-Rad Laboratories</td>
<td>Yes</td>
</tr>
<tr>
<td>Fourth generation</td>
<td>Same mechanism as third generation, but in addition uses a antibody to detect p24 antigen in the patients serum</td>
<td>2 weeks</td>
<td>VIDAS HIV DUO Ultra</td>
<td>Serum/Plasma</td>
<td>HIV-1 gp160, p24 antigen, and peptides representing regions of gp41 from HIV-1 group O and gp36 from HIV-2</td>
<td>bioMerieux Inc.</td>
<td>No</td>
</tr>
</tbody>
</table>

HIV-testing technologies

HIV tests can be characterized as either (1) screening tests or (2) confirmatory tests. The standard testing algorithm is to do a screening test, followed by a confirmatory test for any positive result. Regardless of the type of screening method used, a specimen producing a positive result will be tested again with the same or a different screening test. If that sample is repeatedly reactive, the sample will be assessed with a confirmatory assay. Screening assays, which include the conventional tests (enzyme immunofluorescence assays) and rapid tests, have a high sensitivity for serum antibody and, thus, give few false-negative results. Confirmatory assays include Western Blots (WB), indirect immunofluorescent antibody assays (IFA), and, recently, HIV RNA detection by NAAT. All these tests have a high specificity, meaning that a positive test has a very low probability of being a false positive.

Screening technologies

Enzyme immunoassays (EIA)

The enzyme immunoassay (EIA) is the most common screening test used. It is simple and amenable to high-volume testing, and

Continues on page 14
has high sensitivity and specificity. Many assays are FDA cleared for use in serum, plasma, finger-stick whole blood, oral fluid, and urine. EIAs use an HIV antigen coated on a microwell plate to detect any HIV antibodies present in a specimen. Four generations of EIAs have been produced (see Table 1) with later generations having improved test performance, and shorter window periods during which antibodies cannot be detected. First- and second-generation tests have a window period of about six to 12 weeks for most individuals. Both first- and second-generation tests detect IgG antibodies to HIV. The first-generation test uses viral lysate as a target antigen, while the second-generation tests use recombinant proteins representing HIV capsid and envelope. Currently, second-generation EIAs are the most frequently used HIV-screening tests in the United States.

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Third-generation tests have the ability to detect IgM antibodies in addition to IgG, through the “antigen sandwich method” in which HIV antibodies from the specimen are sandwiched between two antigen molecules, one in the solid phase and one conjugated to an enzyme, such as alkaline phosphatase or horseradish peroxidase. In addition, third-generation tests have the capacity to detect certain HIV subtypes, particularly HIV-1 Group O and HIV-2 which were not included in previous generation tests.

Fourth-generation tests detect IgM and IgG antibodies as well as the presence of the viral capsid antigen p24. The detection of p24 antigen reduces the window period to two weeks, and makes detection of acute HIV infection (that is, HIV infection before seroconversion) possible. Currently, there are eight commercial fourth-generation tests, none of which have been FDA cleared. Fourth-generation tests combine two methodologies into one assay — antigen and antibody detection. Recent studies on fourth-generation tests have compared the sensitivity and window period of various assays, with a focus on the most recent fourth-generation test: VIDAS HIV DUO Ultra (bioMerieux, Marcy-l’Etoile, France). Unlike the other fourth-generation tests, the VIDAS HIV DUO Ultra test produces two distinct results for antigen and antibody detection and has the lowest p24 antigen detection limit at 3 pg/mL. Those characteristics allow the differentiation of acute HIV infection from recent and/or established infections. The microwells of the combined antigen-antibody test are designed differently; the upper part of the well is coated with anti-p24 monoclonal antibodies and the lower part of the well is coated with antibodies that can detect IgG, IgM, and IgA for HIV-1 and -2. The HIV DUO Ultra test was found to be the most sensitive fourth-generation assay (compared to Enzygnost HIV Integral, Enzymun HIV Combi, Genscreen Plus HIV Ag-Ab, and AxSYM HIV Ag/Ab Combo), and was found to be as sensitive as a single p24 assay (Genetic Systems HIV-1 Ag EIA) — making it a viable option for p24 testing.

Screening tests for acute HIV: nucleic-acid amplification testing and fourth-generation EIAs

Acute infections are often missed, as the clinical symptoms associated with acute infection are often absent or subtle, and the standard HIV tests used by clinicians, typically first-generation assays in many developing countries and second-generation tests in the United States, will not identify persons with acute infection. The symptoms, diagnosis, and management of acute HIV infection are beyond the scope of this review and have been recently reviewed elsewhere. Around two weeks into the acute infection, approximately two-thirds of patients have some symptoms attributable to acute retroviral syndrome; the most common symptoms are fever (present in 80% to 90% of patients), malaise, anorexia, myalgias, and headache (in about 50% of patients). Although still controversial, current clinical data suggests that treatment prior to seroconversion may benefit patients in the short term and possibly in the long term by augmenting host immunity and potentially obviating or delaying the need for continuous antiretroviral therapy. Identification of acute HIV infection is also important from a public-health standpoint, as the high levels of viremia, combined with a lack of awareness of infection, make acutely infected individuals a high transmission risk. Mathematical models have suggested that persons with acute HIV infection are the important drivers of the epidemic, and epidemiologic studies suggest persons with recent infection are much more infectious than those who are chronically infected.

In order to diagnose acute HIV infection, it is necessary to detect the presence of HIV antigen in patients that have not yet seroconverted. Therefore, the gold standard for diagnosing acute infection is the use of NAAT in the setting of a negative HIV antibody result. NAAT can be both quantitative and qualitative. Quantitative assays determine the plasma viral load and are used to monitor disease

<table>
<thead>
<tr>
<th>Test name</th>
<th>Specimen type</th>
<th>CLIA category</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Detects HIV</th>
</tr>
</thead>
<tbody>
<tr>
<td>OraQuick</td>
<td>Serum and plasma</td>
<td>Waived</td>
<td>99.3% (98.4-99.7)</td>
<td>99.6% (99.6-99.9)</td>
<td>Yes</td>
</tr>
<tr>
<td>ADVANCE Rapid HIV 1/2 Antibody Test</td>
<td>Plasma</td>
<td>Waived</td>
<td>99.6% (98.5-99.9)</td>
<td>100% (99.7-100)</td>
<td>Yes</td>
</tr>
<tr>
<td>Uni-Gold</td>
<td>Serum and plasma</td>
<td>Waived</td>
<td>100% (99.5-100)</td>
<td>99.7% (99.0-100)</td>
<td>No</td>
</tr>
<tr>
<td>Recombigen HIV</td>
<td>Serum and plasma</td>
<td>Waived</td>
<td>99.8% (98.9-99.9)</td>
<td>99.1% (98.4-99.4)</td>
<td>No</td>
</tr>
<tr>
<td>MultiSpot HIV-1/2</td>
<td>Serum and plasma</td>
<td>Waived</td>
<td>100% (99.9-100)</td>
<td>99.9% (99.6-100)</td>
<td>Yes, differentiates IV-1 from HIV-2</td>
</tr>
<tr>
<td>Clearview HIV 1/2 STAT-PAK</td>
<td>Serum and plasma</td>
<td>Waived</td>
<td>99.7% (98.9-100)</td>
<td>99.9% (99.6-100)</td>
<td>Yes</td>
</tr>
<tr>
<td>Clearview COMPLETE HIV 1/2</td>
<td>Serum and plasma</td>
<td>Waived</td>
<td>99.7% (98.9-100)</td>
<td>99.9% (99.6-100)</td>
<td>Yes</td>
</tr>
</tbody>
</table>

Table 2. Current rapid HIV tests cleared by the U.S. Food and Drug Administration, 2007

Adapted from Health Research and Education Trust; available at http://www.hret.org/hret/programs/hivtransmrpd.htm - Updated August 30, 2007

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Cover Story

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progression and response to antiretroviral therapy. On the other hand, qualitative assays reveal whether HIV RNA is present or not and are used to screen specimens for the presence of HIV antigen. The APTIMA HIV 1 RNA Qualitative Assay (Gen-Probe Inc., San Diego, CA) is the only NAAT cleared by the FDA for (1) diagnosis of primary HIV-1 infection and (2) confirming HIV-1 infection when tests for antibodies to HIV-1 are reactive.

Historically, NAAT has not been included in routine HIV-screening protocols, due to the high cost and labor that most of those technologies require. To decrease the costs, blood-donor programs in the United States have been using pooling algorithms with NAAT. 24 In those algorithms, antibody-negative specimens are combined in pools, and each aggregated screening pool is assessed by NAAT. A negative pool ends the screening protocol. If a pool has a positive NAAT result, then the pool is deconstructed further into either smaller intermediate pools or individual specimens until the positive specimen is identified. The primary benefit of specimen pooling is significantly decreased costs compared to testing each individual specimen. The major drawback is that pooling involves the dilution of specimens, which may impact test sensitivity. Recent public-health efforts to diagnose acute infection have led to the use of pooling with NAAT in routine HIV screening in certain settings.18-20

Experiences thus far with the pooling technique for routine testing have been very encouraging, particularly among traditional high-risk populations. Currently, pooled NAAT is being used in public HIV-testing sites in Los Angeles, San Francisco, North Carolina, New York City, Maryland, Washington, DC, Florida, and Seattle-King County. At the public STD clinic in San Francisco, routine screening for acute infection using NAAT resulted in an increase in HIV case detection of 8.8%;24 and in the state of North Carolina, the addition of NAAT increased the number of cases identified by 3.9%.19 The study in North Carolina and other studies on the pooled technique in community-screening programs found the protocol cost effective. In North Carolina, the testing cost only increased by $3.63 per specimen.

Currently, sampling-pooling protocols differ in terms of the size of the master pools, the number of intermediate pools, and the type of nucleic-acid amplification test used. Limited data exist on the comparative performance of NAAT tests for the screening of pooled specimens, but tests with lower detection limits are likely to perform better. Further studies are warranted to assess the comparative performance of different NAAT tests for use with pooled samples.22

While experiences with NAAT seem promising, the optimal approach to detect HIV infection in conventional settings is still being defined.23 To date, NAAT has been incorporated into screening protocols primarily in settings that test high-risk individuals. The cost-effectiveness, and positive and negative predictive value in NAAT in low-risk settings is unclear. Third- and fourth-generation EIAs may be an alternative to NAAT in certain settings, as they can identify some acute infections, although less than the number of acute infections identified by NAAT. For example, in Seattle-King County, HIV-RNA screening for acute HIV infections in 2003 revealed seven acute infections via NAAT. Four of the seven samples were available for re-testing, and two of the four specimens were found to be positive with a third-generation EIA.24 A prospective study in Malawi found that parallel rapid and p24-antigen testing detected approximately 90% of acute HIV cases identified by NAAT.25 While third- and fourth-generation EIAs are likely to provide an alternative for acute HIV detection in low-income settings, algorithms combining standard EIAs followed by pooled-specimen screening by a NAAT are recommended for settings in which resources are available.

**While the benefits of home testing are compelling, there are still many outstanding questions.**

**Rapid HIV antibody tests**

One disadvantage of standard EIAs is that it can take up to two weeks for patients to get results, and many patients in publicly funded testing sites do not return to get their test results. Lack of follow up for test results is a significant problem, and failure to return for test results has been found to occur more frequently among individuals at elevated risk of contracting HIV and individuals who tested positive.26 Rapid HIV-test results can be received the same day, as testing takes less than 30 minutes to complete, and the test can be done at both clinical and non-traditional sites, such as emergency departments, community centers, and health fairs. In general, rapid tests are preferred by patients in comparison to conventional EIAs, and those tested are more likely to receive their results — especially at non-traditional sites such as needle exchanges or bathhouses.27,28

There are four rapid tests cleared for HIV-1/2 detection, one of which, the OraQuick Advance Rapid HIV-1/2 Antibody Test, is cleared for testing of oral fluid (see Table 2). The sensitivity of the tests is comparable to standard second-generation EIA testing.28 Rapid tests each have a synthetic antigen (the gp41 region of HIV-1, and gp36 for HIV-2) affixed to a test membrane, and a sample (finger-stick blood, venipuncture blood, or oral fluid) is applied to the membrane. If the sample contains antibodies to the gp41 region of HIV, then the membrane will change color. In addition, each test has a goat anti-human IgG antibody for control, and each test requires the periodic use of external controls.

Each rapid test has an assigned Clinical Laboratory Improvement Amendments of 1998 (CLIA’98) category that determines the personnel and the type of facilities required to perform the test. Persons without formal laboratory training and outside the traditional laboratory can perform waived tests. To classify as a CLIA-waived test, the test must use direct, unprocessed specimens (such as oral or whole blood), and must be easy to perform by persons without formal laboratory training. The rapid test is the only HIV-testing modality that can be done outside of the laboratory setting.

Another benefit of the rapid test is that it can be non-invasive, as oral-fluid rapid testing was FDA cleared in 2004. Post-marketing surveillance of the Ora Quick Advance Rapid HIV 1/2 Antibody Test on whole blood and oral fluid yielded favorable results.29 Testing of over 135,000 whole-blood samples and over 26,000 oral-fluid tests yielded a specificity of 99.98% with a positive predictive value of 99.24% for blood, and specificity of 99.89% with a positive predictive value of 90% for oral fluids. While oral fluid was slightly less specific, experts do not discourage its use, as the increased acceptance of the non-invasive method will likely outweigh the small deficit in specificity.29

While data on rapid HIV testing are encouraging, their use still requires some caution. Steckler, et al,26 reported three cases of early infection that were missed by OraQuick Rapid HIV-1
Rapid HIV testing at home has the potential to increase access to HIV testing at home. Still under debate and development: urine tests

Urine HIV tests measure intact HIV IgG antibodies found in urine specimens and have been cleared by the FDA for use with EIA and Western Blot. Assays using urine have the potential to reduce barriers to testing, as they are simple and non-invasive, and the urine can be stored for long periods at room temperature. Despite its advantages, the urine-based HIV test is not commonly used, although test performance may be similar to blood-based testing (in one study the Maxim Urine HIV-1 EIA had a sensitivity of 98.7%).

Rapid urine tests, although commercially available, are not FDA cleared. The Aware-Urine assay (Calypte Biomedical Corp., Rockville, MD), initially had promising preliminary results, but subsequently, found to be positive by third-generation EIAs and Western Blots. Although the cases may have been missed due to operator error, those findings support the continued use of RNA pooling within rapid-testing protocols in high-risk populations. Case reports of false-negative test results also emphasize the importance of clinical judgment in the interpretation of negative results.

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Still under debate and development: urine tests

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Rapid urine tests, although commercially available, are not FDA cleared. The Aware-Urine assay (Calypte Biomedical Corp., Rockville, MD), initially had promising preliminary results, but the sensitivity was found to be poor in a recent study set in rural Uganda. The study reported that 942 urine samples yielded a sensitivity of 88.7% and specificity of 99.9% in comparison to EIAs using serum confirmed by Western Blot.

HIV testing at home

Rapid HIV testing at home has the potential to increase access to testing, but its role in public health and testing protocols is still unclear. Benefits of home testing are the perception of increased privacy and ease — as the test eliminates the need to attend a publicly funded HIV-testing site or medical provider. The FDA has not yet cleared a complete HIV home-testing kit; however, in 1999, it cleared the first HIV home sample-collection kit — the Home Access HIV-1 Test System. The collection kit allows individuals to mail in a sample of whole blood that was collected at home by finger stick and obtain results by telephone three days later.

The FDA reviewed testimony in 2006 regarding the clearance of a complete rapid HIV home test, the OraQuick ADVANCE 1/2. This test allows individuals to use either finger-stick whole blood or oral fluid as a specimen. The specimen is placed on the test device, and the test device is then placed into a solution vial. Test results are available 20 to 40 minutes after putting the test vial into the developer solution. A purple test line with a positive control line indicates a positive test, whereas a negative test will show only the control line (Oraquick Advance Rapid HIV 1/2 Antibody Test package insert: Orasure Technologies Inc., Bethlehem, PA). While the benefits of home testing are compelling, there are still many outstanding questions. Current concerns surround the cost; for example, laboratories pay $12 to $17 for each OraQuick kit, and the cost will likely increase for over-the-counter sales. A high cost may make the test unattainable by many high-risk populations and may deter its use by the general public. In addition, reports of unexplained high rates of false-positive OraQuick rapid-test results in a few publicly funded HIV-testing sites raised concerns about the potential for false-positive results in the home setting. Additional questions regarding complete home HIV-testing kits are well summarized by Branson: 1) Do home HIV tests expand access to testing? 2) Can consumers correctly use the home HIV test? 3) Do home HIV-test users experience potential harm because they receive no face-to-face counseling? 4) How do home sample-collection kits affect public-health practices? Given the many unknowns, the FDA has required that the OraQuick manufacturers — at a minimum — demonstrate the device is accurate in the hands of lay users prior to FDA clearance; currently phase II trials are underway.

Confirmatory HIV tests

All positive screening tests must be confirmed by a confirmatory test, either a WB or indirect immunofluorescent antibody assay, and most recently the APTIMA HIV 1 RNA Qualitative Assay was the first nucleic acid amplification test to be cleared by the FDA for confirmatory testing. Confirmatory tests are highly sensitive and specific, and they provide a definitive diagnosis of HIV infection. The confirmatory test is used to confirm a positive screening test result, and it is not intended to be used to screen for HIV infection. The confirmatory test is typically performed on a blood sample, but it can also be performed on saliva samples, which may provide a more convenient and accessible testing option for certain populations.
31. Trust HFAE. FDA-Approved HIV-1/2 Enzyme-Linked Immunosorbent Assays (ELISA) or ELISA. 2006.
Sex, human papilloma virus infection, and head and neck cancer

By Kyle Bernstein, PhD, ScM, and Jeffrey D. Klausner, MD, MPH

Over 100 human papillomavirus (HPV) types have been identified, with many linked to cancer. The burden of head and neck cancers is relatively small; it is estimated that 34,360 new cases of head and neck cancer will have been diagnosed in 2007 in the United States, and 7,550 deaths associated with head and neck cancers will occur.¹ Head and neck cancers account for less than 3% of new cancer diagnoses and 1.3% of cancer-related mortality.¹ Although molecular evidence supports the causal role of HPV in squamous-cell carcinomas of the head and neck, epidemiologic data showing an association between HPV and those cancers are lacking. A recent case-control study by D’Souza, et al.,² adds an epidemiologic perspective to the growing body of scientific literature supporting the role of HPV infection in head and neck cancers.

In D’Souza, et al.,’s study, characteristics of patients with head-and-neck squamous-cell carcinoma diagnosed in the Johns Hopkins Hospital otolaryngology clinic between 2000 and 2005 were compared with those without a history of cancer seen at the same clinic during the same period. Enrolled patients submitted oral saline rinse, oral mucosal brush, and serum specimens. Researchers used multiplex polymerase chain reaction (PCR) assays targeting the L1 region of HPV to determine the HPV type(s) in tumor specimens, when available. Additionally, they used an enzyme-linked immunosorbent assay (ELISA) to measure serum antibodies to HPV-16 (the HPV subtype most commonly associated with head and neck cancers) L1 protein, and E6 and E7 proteins. The authors use multivariable regression to adjust for age, sex, smoking, alcohol use, dentition, dental-hygiene practices, and family history of head and neck cancers. To elucidate possible pathways in the etiology of head and neck cancers, various statistical interactions among smoking, alcohol use, and HPV infection were explored.

Enrolled cases (n=100) and controls (n=200) were primarily male (86%), less than 65 years old (85%) and white (86%). The authors found increasing numbers of vaginal and oral sex partners, having had a casual sex partner and never or rarely using condoms, were significantly associated with an increased likelihood of head and neck cancer. When analysis of sexual behaviors was restricted to only head and neck cancers that harbored HPV-16, those associations were strengthened. Having had a same-gender sex partner or a sex partner with a history of an HPV-related cancer was not associated with head and neck cancer.

Continues on page 22
Participants who smoked or drank alcohol were more likely to have head and neck cancer. This increased risk from tobacco and alcohol use existed when the analyses were restricted to those who had no evidence of HPV-16 infection. The associations between alcohol use and smoking, and head and neck cancer, however, were no longer found among participants who had HPV-16 infection, suggesting that tobacco and alcohol use may be important factors in head-and-neck-cancer development in the absence of HPV-16 infection but may be less important among those who have been infected with HPV-16.

Head and neck cancers were also strongly associated with HPV-16 L1 seropositivity, HPV-16 E6 or E7 seropositivity, oral HPV-16 infection, and any oral HPV infection. HPV-16 DNA was recovered from 72% of the paraffin-embedded head-and-neck-cancer specimens.

This study is important for several reasons. It confirms the findings of other observational studies providing important epidemiologic evidence to complement the basic science data suggesting an association between HPV (and more specifically HPV-16) in head-and-neck carcinogenesis. Additionally, the findings of this study suggest that oral HPV infections may be sexually acquired. A strong and consistent dose response was seen with increasing numbers of oral sex partners and increased likelihood of head and neck cancer. Associations between oral sexual activity and cancer were only strengthened when the analysis was limited to HPV-16-positive head and neck cancers. Given the relative rarity of head and neck cancer, and the high frequency of oral sex — from a public-health standpoint — curtailing the frequency of oral sex is not likely a practical strategy to reduce the risk of head and neck cancer.

The authors reported an independent association between having a family history of head and neck cancer, and a new diagnosis of head and neck cancer. Family clustering of cancer has been reported by others, and similar findings have been noted with respect to cervical cancer. It remains unclear whether that finding reflects a genetic component to HPV-associated cancers and/or shared environmental exposures, or whether it is spurious. Furthermore, this case-control study found associations between poor oral hygiene and head and neck cancers. Given that many dental problems are a result of bacterial infections, the authors suggest that bacterial co-infection may play a role in the development of head and neck cancers, similar to the relationship between Chlamydia trachomatis and cervical cancers. Finally, D’Souza, et al, reported that while tobacco and alcohol use may be important risk factors for head and neck cancers, their data suggested no synergistic effect with HPV infection. In short, two pathways may be involved in the development of head and neck cancer — one driven by HPV infection and one by tobacco and/or alcohol use.

As with any study, its limitations must be considered. Since this was a case-control study, no claims can be made regarding causality. Moreover, the cases and controls were selected from the patient population of the Johns Hopkins University otorlaryngology clinic, a tertiary-care specialty clinic that draws its patient population from many states and countries. As a result, the patients enrolled may not represent the “typical” head-and-neck-cancer patient who may have been seen at his local otolaryngology clinic, and the controls may also represent patients with atypical complaints and characteristics.

This study by D’Souza, et al, adds to the growing body of literature highlighting the importance of HPV infection in cancers other than cervical. These data also suggest the potentially limited effect of factors, such as alcohol and smoking, in head-and-neck-cancer development, in the absence of HPV infection. Although multitudinous authorities encourage condoms and other barriers for oral sex, data suggest that few use them. Therefore, developing behavioral interventions for head and neck cancer may not be feasible. Compared to other HPV types, HPV-16 is disproportionately associated with head and neck cancers, and is one of the four types of HPV included in the recently licensed Gardasil HPV vaccine. Yet, the potential effectiveness of Gardasil for prevention of oral HPV infection and subsequent cancer development will not be well characterized for many years.  

Kyle Bernstein, PhD, ScM, and Jeffrey D. Klausner, MD, MPH, are both affiliated with the STD Prevention and Control Section of the San Francisco Department of Public Health.

References

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