Deborah Chute, MD (r), pictured here with Ghada Aramouni, CT(ASCP), believes that HC2 testing is a cost-effective alternative to CISH for high-risk HPV testing in squamous cell carcinoma.

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Cover Story

High-Risk HPV Testing in Squamous Cell Carcinoma by HC2

By Deborah Chute, MD

Head and neck squamous cell cancer (SCC) is a significant public health problem worldwide with half-a-million cases diagnosed annually. These cancers frequently are asymptomatic in their early stages so that presentation is often late and outcomes are poor.

A subset of head and neck SCC, representing approximately 35 percent of all head and neck SCC, is related to high-risk human papilloma virus (HPV) infection. HPV-related SCCs have important prognostic and treatment differences from non-HPV-related SCC. Recent studies confirm that HPV-positive oropharyngeal tumors comprise a distinct clinical and pathological entity that is associated with improved five-year survival. In one study, overall survival at five years was 53% for HPV-positive tumors compared with 31% in HPV-negative tumors. This improved survival is believed to be related to increased radio- and/or chemotherapy sensitivity of HPV-related tumors.

Benefits of Determining HPV Status

Many patients with HPV-related SCCs of the head and neck present with neck metastasis, which most often is diagnosed by fine needle aspiration (FNA) biopsy. Historically, a surgical biopsy of the primary tumor, usually an oropharyngeal lesion, is required to determine HPV status.

In patients with a known oropharyngeal mass, knowledge of HPV status using FNA material would avoid additional surgical biopsies strictly for HPV testing. In addition, the presence of HPV in a neck metastasis may direct clinicians to identify an occult oropharyngeal primary lesion.

Finally, in 15 to 20 percent of patients presenting with metastatic SCC to the neck, a primary head and neck mucosal SCC is never found because of its small size. In these patients, the FNA sample is the only material available for HPV testing to direct treatment recommendations. These patients stand to benefit the most from an alternative to surgical biopsy for evaluation of HPV status in that they could potentially avoid whole-head radiation.

An Alternative to Surgical Biopsy

Clearly, a test to detect HPV in cytology specimens with high specificity and sensitivity has the potential to direct patient care and avoid the need for additional surgical biopsies. However, the optimal testing for HPV detection in head and neck SCC remains controversial. Most work has focused on HPV detection in surgical resection tissue. Methods used have included polymerase chain reaction (PCR) for HPV, chromogenic in situ hybridization (CISH) and p16 immunohistochemical staining (a surrogate marker for HPV).

The Hybrid Capture 2 (HC2) test is the most commonly used test for detecting high-risk HPV in uterine cervix samples acquired during Pap smears. This test currently is the standard of care in Pap test high-risk HPV testing but cannot be used in formalin-fixed, paraffin-embedded tumor samples such as the ones shown.

Squamous cell carcinoma of the tonsil with positive HPV testing

Top: HPV CISH stain, 20x (cell block preparation)
Bottom: H&E stain, 20x (cell block preparation)
as those obtained in a surgical resection. However, the HC2 test can be used to test FNA samples from head and neck SCC that are rinsed in a methanol-based fixative (CytoLyt® or Preservcyt®, both Cytyc, Boxborough, Mass.).

Study Design

We are comparing the utility of HC2 with cell block CISH for detecting clinically relevant high-risk HPV in metastatic SCC FNA sample rinses.

The study draws from the Cleveland Clinic head and neck cancer population with a goal of enrolling 60 to 100 patients. Patients are identified through a weekly CoPath search to find FNA samples with a diagnosis of SCC or atypical cells, cannot exclude SCC. After the initial diagnosis is completed on each case, any excess FNA material is stored in a methanol-based fixative and submitted for HC2 testing. Additionally, when sufficient material is available, a cell block is made.

HC2 testing employs in vitro nucleic acid hybridization with signal amplification using microplate chemiluminescence to detect HPV subtypes 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, and 68. This list includes 95 percent of all HPV subtypes that cause cancer.

Cell blocks from the study population undergo high-risk HPV CISH testing, which detects high-risk HPV subtypes 16, 18, 31, 39, 45, 51, 52, 56, 58, 66. Additionally, testing for p16 and HPV CISH is performed on a formalin-fixed, paraffin-embedded block of the surgical biopsy/resection specimen as the gold standard.

Results for all tests are reported as positive or negative.

Encouraging Preliminary Results

Enrollment in the study is ongoing. Over eight months, 32 patients had a head and neck FNA with a diagnosis of SCC or suspicion for SCC. A total of 21 (66%) had formalin-fixed, paraffin-embedded surgical material available for HPV testing as the gold standard. Of this subgroup, the primary sites were tonsil (11), pyriform sinus (1), base of tongue (1), larynx (1), skin (2), lung (1), and unknown (2). HC2 testing was performed on 31 samples, and CISH HPV testing was performed on the cell block for 23 samples.

On the surgical specimens, 11 patients (53%) were HPV CISH-positive, all of whom had primary SCC of the tonsil. Results of the HC2 testing and CISH HPV testing on the corresponding FNA cytology specimens are displayed in Tables 1 and 2, respectively. The sensitivity of HC2 and cell block HPV CISH for the detection of high-risk HPV was 60% and 66%, respectively. The specificity of HC2 and cell block HPV CISH in detecting high-risk HPV was 100% and 85%, respectively.

Only 11 patients had sufficient material for all three tests (HC2, cell block CISH and surgical specimen testing), but HC2 performed consistently better than CISH HPV testing in this group. (Table 3).

<table>
<thead>
<tr>
<th>Table 1: Comparison of HC2 with Surgical HPV CISH</th>
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<tr>
<td>Surgical HPV CISH</td>
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<tr>
<td>Positive</td>
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<tr>
<td>Positive</td>
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<tr>
<td>Negative</td>
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<th>Table 2: Comparison of Cell Block HPV CISH with Surgical HPV CISH</th>
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<tr>
<td>Surgical HPV CISH</td>
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<tr>
<td>Positive</td>
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<tr>
<td>Positive</td>
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<tr>
<td>Negative</td>
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<tr>
<th>Table 3: Comparison of HC2 with Cell Block HPV CISH with Surgical HPV CISH in Patients with All Three Tests</th>
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<tbody>
<tr>
<td>Surgical HPV CISH</td>
</tr>
<tr>
<td>Positive</td>
</tr>
<tr>
<td>Positive (n=4)</td>
</tr>
<tr>
<td>Negative (n=7)</td>
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</table>

Summary

HC2 shows similar sensitivity to HPV CISH (60% vs. 66%) and better specificity (100% vs. 85%). HC2 testing can be performed on limited samples, although negative results in this setting should be interpreted with caution as there is a risk of false negative results. Considering HC2’s acceptable sensitivity, high specificity and modest cost compared with HPV CISH, it represents a reasonable alternative for HPV testing.

These preliminary data suggest that this new application of a familiar, widely available testing method can be cost-effective and clinically useful. The ability to test FNA material for high-risk HPV has the potential to open new avenues for the use of cytology in the diagnosis and management of patients with head and neck SCC.
The Role of ERG/P63 Double Immunohistochemical Staining in the Diagnosis of Limited Cancer in Prostate Needle Biopsies

By Oksana Yaskiv, MD, Xiaochun Zhang, MD, PhD, Kelly Simmerman, MS, Tom Daly, MD, Huiying He, MD, PhD, Sara Falzarano, MD, Longwen Chen, MD, PhD, Cristina Magi-Galluzzi, MD, PhD, Ming Zhou, MD, PhD

Background

The widespread use of prostate-specific antigen (PSA) screening has led to detection of a growing number of early-stage, low volume prostate adenocarcinoma (PCa). In this setting, immunohistochemistry (IHC) is an invaluable tool for working up difficult prostate biopsies. The most common IHC markers currently in clinical use include basal cell markers and alpha-methyl-acyl-CoA (AMACR, P504S). However, none of these markers is 100 percent sensitive or specific for the diagnosis of PCa.

A PCa-specific tumor marker that is not expressed in non-cancerous lesions would unequivocally identify PCa in prostate biopsies. The recently discovered TMPRSS2:ERG gene rearrangement may be such a candidate marker. ERG gene rearrangement is present in approximately 50 percent of PSA-screened radical prostatectomy cohorts and is highly prostate cancer-specific as it is identified only in PCa and occasionally in high-grade prostatic intraepithelial neoplasia (HGPIN) intermingled with ERG-positive cancer glands.

Until recently ERG rearrangement status could be assessed only by fluorescence in situ hybridization (FISH). Recently, two studies reported two novel anti-ERG monoclonal antibodies. Positive IHC staining with these two antibodies has been found to highly correlate with TMPRSS2:ERG gene rearrangement. Given the ease of performing IHC versus FISH, pathologists are enthusiastic about the prospect that ERG IHC may be useful for prostate biopsy evaluation.

In the Cleveland Clinic Pathology & Laboratory Medicine Institute, we have developed an antibody cocktail containing ERG and prostate basal cell marker P63 antibodies and assessed the utility of this ERG/P63 cocktail in the diagnosis of limited PCa in prostate needle biopsies. We addressed three main issues:

1) the frequency of ERG-positive status in prostate biopsies with limited cancer
2) the relative occurrence of ERG in non-cancerous glands in prostate biopsy and
3) the advantages of using the ERG/P63 cocktail in prostate biopsies compared with the use of basal cell markers alone and/or of AMACR.
Validation of ERG/P63 Double Immunohistochemical Staining

We performed double immunohistochemical stains for ERG and P63 on 73 prostate biopsies with limited cancer (cancer involving only one core out of the entire biopsy set and occupying <1 mm of the involved biopsy core). Using the vascular endothelial cells as the internal positive control to which we assigned a staining score of “strongly positive,” the expression of ERG protein in PCa and other glands was scored as negative, weak, moderate, or strong.

ERG staining was positive in 29 of 73 (40%) prostate biopsy cases, including 20 (69%) scored as strong positive, five (17%) as moderate positive and four (14%) as weak positive. ERG staining was uniform in 25 (86%) of ERG-positive cases with uniform staining intensity in >90% of the cancer cells. Four cases (14%) had heterogeneous ERG staining patterns.

The Gleason score (GS) for the limited PCa was 6 in 61 cases, 7 in 11 cases and 8 in one case. ERG was positive in 26 (43%) of PCa cases with GS 6 and in three (25%) of PCa cases with a Gleason score ≥7 (p= 0.345 by Fisher’s exact test).

HGPIN was present in 14 of 73 cases, and three cases (21%) of HGPIN were positive for ERG protein expression. All the positive HGPIN glands were intermingled with or immediately adjacent to and within < 0.1 mm of the ERG-positive cancer glands. In three cases (4%), positive ERG staining was found in morphologically benign glands immediately adjacent to or intermingled with ERG-positive PCa glands. The non-cancerous lesions identified in the biopsies, including simple atrophy in eight cases, partial atrophy in 34 cases and a combination of both in 26 cases, were all negative for ERG staining. No benign prostate glands distant from the PCa were positive for ERG.

Role of ERG/P63 Double Immunostain

Our results are consistent with published studies that ERG gene rearrangement was found only in PCa and the approximately 20 to 25 percent of HGPIN that was intimately associated with ERG-positive PCa. Only rarely was ERG expression found in benign glands. Furusato et al estimated that <0.001% of benign glands were positive for ERG IHC when radical prostatectomy specimens were examined. Therefore, the specificity of ERG for PCa is more than 99.99%.

Currently available data suggest that a positive ERG IHC is almost always seen in PCa or HGPIN glands that are associated with PCa. Therefore, positive ERG IHC suggests that the ERG-positive glands are either cancerous glands or in close proximity to cancerous glands. ERG protein expression can be very helpful in the workup of prostate biopsies, and positive staining supports a cancer diagnosis. However, due to its sensitivity (40-50%), a negative ERG IHC does not rule out PCa.

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The advantages of using double immunostaining for both ERG and P63 in the prostate biopsy evaluation include its suitability for the minute focus of atypical or cancerous glands that may be present in only one section and simultaneous evaluation of ERG and P63 in the same prostate gland. The most significant advantage of the ERG/P63 cocktail is the high sensitivity of P63 and high specificity of ERG that it offers. Except for rare P63-positive PCa in which cancer cells are diffusely positive for P63 and where the staining pattern is different from benign glands, all PCa lacks basal cells markers expression, including P63. Therefore, the sensitivity of P63 for PCa is 100%. On the other hand, ERG is highly specific for PC with specificity approaching 100% in published studies. The likely immunophenotype and corresponding diagnosis are shown in the Table below.

**ERG/P63 Immunophenotype and Anticipated Diagnosis**

<table>
<thead>
<tr>
<th>Immunophenotype</th>
<th>Diagnosis</th>
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<tr>
<td>ERG+/P63-</td>
<td>Cancer</td>
</tr>
<tr>
<td>ERG+/P63+</td>
<td>Non-cancer in close proximity to cancer</td>
</tr>
<tr>
<td>ERG-/P63-</td>
<td>Cancer or benign mimickers of cancer</td>
</tr>
<tr>
<td>ERG-/P63+</td>
<td>Non-cancer</td>
</tr>
</tbody>
</table>

**Summary**

We have demonstrated the diagnostic utility of ERG/P63 double stain as an immunohistochemical marker in the diagnosis of limited PCa in prostate needle biopsies. ERG is expressed in 40% of limited cancers in prostate biopsies. It also is expressed in a subset of high-grade PIN and benign glands, all of which are intermingled or immediately adjacent to cancer. It is not expressed in other benign lesions. The cocktail containing P63 and ERG provides high sensitivity for P63 and high specificity for ERG and is potentially useful in the workup of difficult prostate biopsies.

**References**

Dr. Yerian Lectures on Liver Disease to Ohio Path Society

Lisa Yerian, MD, Section Head, Surgical Pathology, Department of Anatomic Pathology, delivered the afternoon program at the Ohio Society of Pathologists Winter Meeting in Columbus, Ohio, January 29. Dr. Yerian, who is Director of Hepatobiliary Pathology in the Pathology & Laboratory Medicine Institute, presented “Updates in Fatty Liver Disease: Interpretations and Implications” followed by “Liver Tumors: Diagnosis of Primary and Metastatic Lesions.”

In her first presentation, Dr. Yerian reviewed the latest research and advances in fatty liver disease, including a discussion of liver biopsy as a diagnostic tool for steatohepatitis. In her second lecture Dr. Yerian focused on the accurate diagnosis of nonhepatocytic tumors, a rare class of liver tumors. (See slides, r.)

These topics are of particular relevance, Dr Yerian noted, because fatty liver disease is the most prevalent type of liver disease in the world across all age groups and affects approximately 30 percent of all U.S. adults.

Anatomic Pathology Faculty to Present at USCAP

Department of Anatomic Pathology staff members will present a total of nine courses and participate in numerous poster and platform sessions at the United States and Canadian Academy of Pathology (USCAP) 2011 Annual Meeting. The meeting takes place February 26 through March 4 in San Antonio, Texas. Additionally, department staff will moderate three companion society meetings, participate as panelists at two evening specialty conferences and moderate three scientific platforms.

Participating faculty include Valeria Arrossi, MD; Ana Bennett, MD; Steven Billings, MD; Chris Booth, MD; Longwen Chen, MD; James Cook, MD, PhD; John Goldblum, MD; Donna Hansel, MD, PhD; Eric Hsi, MD; Cristina Magi-Galluzzi, MD, PhD; Thomas Plesec, MD, Richard Prayson, MD; Jordi Rowe, MD; Brian Rubin, MD, PhD; and Ming Zhou, MD, PhD.

Dr. Goldblum, Chair, Anatomic Pathology in the Cleveland Clinic Pathology & Laboratory Medicine Institute, chairs the USCAP Education Committee. Continuing a long tradition of PLMI involvement in USCAP professional education activities, Drs. Cook, Hansel, Hsi, Magi-Galluzzi and Zhou and Carol Farver, MD; Wally Henricks, MD; Aaron Hoschar, MD; Rish Pai, MD; Thomas Plesec, MD; Richard Prayson, MD; Rene Rodriguez, MD; Andres Roma, MD; Carmela Tan, MD; and Lisa Yerian, MD; also serve on the USCAP Abstract Review Board.
Deborah Chute, MD, is an associate staff member in the Department of Anatomic Pathology. Her specialty interests include cytopathology and head and neck and endocrine pathology.

A graduate of the University of Pennsylvania School of Medicine, Dr. Chute completed a residency in pathology at the University of Virginia Medical Center followed by a fellowship in surgical pathology at Stanford University Hospital. She completed her advanced training with a fellowship in cytopathology at the University of Virginia Medical Center. She has published nearly three dozen papers in her specialty areas.

Dr. Chute was appointed to the Cleveland Clinic staff in 2009. She can be reached at 216.444.0291 or chuted@ccf.org.

Ming Zhou, MD, PhD, is a staff pathologist in the Department of Anatomic Pathology and an assistant professor at Cleveland Clinic Lerner College of Medicine of Case Western Reserve University. He is board-certified in anatomic and clinical pathology.

Dr. Zhou’s special interest is in genitourinary disease, and his research interest is in the development of novel diagnostic and prognostic markers for genitourinary malignancies.

He received his medical degree from Fudan University Medical School in Shanghai, China and his doctorate from the University of Cincinnati College of Medicine in Cincinnati, Ohio. He completed a residency and fellowship in pathology at the University of Michigan, and a fellowship in kidney-urologic pathology at the Johns Hopkins Hospital.

Dr. Zhou can be reached at 216.445.3829 or zhoum@ccf.org.