American Head and Neck Society - Journal Club
Volume 15, September 15, 2017

PREVENTION & EARLY DETECTION COMMITTEE EDITION

*This Issue of the AHNS Journal Club has been compiled and reviewed by members of the **AHNS Prevention & Early Detection Committee:***

Cherie-Ann Nathan, MD FACS (Chair)
Mihir Bhayani, MD FACS
Ann Gillenwater, MD
Zhen Gooi, MD
Andrew Nemechek, MD
Andrew Tassler, MD

Table of Contents** – **click the page number to go to the summary and full article link.

Analysis of Plasma Epstein–Barr Virus DNA to Screen for Nasopharyngeal Cancer

K.C. Allen Chan, John K.S. Woo, Ann King, Benny C.Y. Zee, W.K. Jacky Lam, Stephen L. Chan, Sam W.I. Chu, Constance Mak, Irene O.L. Tse, Samantha Y.M. Leung, Gloria Chan, Edwin P. Hui, Brigette B.Y. Ma, Rossa W.K. Chiu, Sing-Fai Leung, Andrew C. van Hasselt, Anthony T.C. Chan and Y.M. Dennis Lo.

*From **New England Journal of Medicine**, August, 2017*

Background/Methods: In endemic regions of the world, undifferentiated type nasopharyngeal cancer (NPC) occurs at significantly higher rates (up to 35/100,000 in middle aged men) than elsewhere. In these patients Epstein-Barr Virus (EBV) has been shown to be closely associated with development of NPC and circulating DNA is used as a tumor marker in these patients with high sensitivity (96%) and specificity (93%). Most currently used tumor markers are proteins generated by the tumor (for example PSA) and it is unknown whether small NPC tumors will generate or release significant EBV DNA for detection.

The majority of NPC is diagnosed at advanced stage once it becomes symptomatic from the primary tumor or nodal disease is apparent. Earlier stage diagnosis would yield improved oncologic and survival outcomes but the tumor lacks symptoms at this early stage in most instances. With this in mind, a prospective trial of more than 20,000 ethnically Chinese men between ages 40-62 was carried out in Hong Kong with the goal of assessing circulating EBV DNA as a screening test for NPC within an endemic population.

Results: 20,349 participants were identified through public health education sessions and their blood was obtained to evaluate for circulating EBV DNA. After exclusions, 20,174 patients were evaluable. 1112 (5.5%) had detectable EBV DNA and these patients were then retested ~ 1 month later to see if they had continued detectable viral DNA. 309/1112 (27.8% of those initially positive, 1.5% of all participants) had continued detectable EBV DNA. 300 of these patients underwent endoscopic examination and 275 underwent MRI in addition to endoscopic exam. 34/300 (11%) were diagnosed with NPC. 3 patients did not have visualized tumor on exam which was identified on MRI.

Compared to an historical cohort, study participants were more likely to have stage I and II disease (70% vs. 20%). Only one patient had disease recurrence. 3-year progression free survival among those with NPC detected by EBV DNA screening was 97%, compared to 70% in the historical control group. The sensitivity and negative predictive value of this screening protocol were 97.1% and 99.995% respectively. Its specificity was 98.6%.

Discussion: Recently, "liquid biopsy" techniques, the analysis of circulating DNA derived from cancer cells, have been evaluated with regard to diagnosis, treatment selection, and monitoring for recurrence after treatment. Little is known, however, about the use of circulating DNA as a screening method applied across at-risk populations. Following earlier proof-of-principle work, Chan, et al.demonstrate in this prospective cohort study the feasibility of screening asymptomatic patients for circulating EBV DNA. The authors used both nasal endoscopy and MRI to corroborate circulating DNA findings. Combining these modalities maximized the investigators' ability to detect the presence of disease *and* enabled a robust analysis of the plasma DNA assay's performance to do the same. The authors demonstrate that small tumors do indeed elute enough DNA for detection and analysis. Their discussion of EBV DNA clearance and, hence, turnover rate of nasopharyngeal carcinoma cells, reveals the remarkable propensity of these tumors to rapidly grow, even the tumors in this population of many early-stage patients.
The investigators discuss two sources of bias that can affect the reported improvement in progression-free survival in the study cohort: length-time and lead-time bias. They conjecture that the possibility of length-time bias is small as NPC normally possesses a biology that results in early disease progression and metastasis. Similarly, they posit that lead-time bias is minimized as the improvement in survival is more likely to be the result of early delivery of effective treatment compared to the effect of early diagnosis only.

One shortcoming of this study is the relatively short follow-up interval (22 months).
In closing, Chan and coauthors have demonstrated that screening asymptomatic patients for circulating EBV DNA is technically feasible and contributes to improved oncologic outcome measures in a large at-risk population.

back to top

Nomogram for risk prediction of malignant transformation in oral leukoplakia patients using combined biomarkers

Zhang X, Kim KY, Zheng Z, Bazarsad S, Kim J.

*From **Oral Oncology**, September 2017*

OBJECTIVE: Squamous cell carcinomas (SCC) are the most common malignancies in the oral mucosa; these carcinomas have been preceded by potentially malignant oral disorders (PMODs), mostly oral leukoplakia (OL). No specific biomarker has been widely accepted for predicting the risk of malignant transformation of PMODs. The aim of this study was to develop an accurate prediction model for the malignant transformation of OL using clinical variables and candidate biomarkers.

MATERIALS AND METHODS: To achieve this goal, 10 candidate biomarkers that had previously been reported as useful molecules were investigated: P53, Ki-67, P16, β-catenin, c-jun, c-met, insulin like growth factor II mRNA-binding protein (IMP-3), cyclooxygenase (COX-2), podoplanin (PDPN) and carbonic anhydrase 9 (CA9). For this study, malignant transformed (n=22, median interval of malignant conversion: 3.3years) and untransformed (n=138) OL specimens with median follow-up period of 11.3years (range: 4.6-23.2years) were immunohistochemically stained.

RESULTS: Using univariate Cox regression analysis, all biomarkers were proven to be significant for predicting malignant transformation in OL. To reach the highest prediction accuracy, the repeated simulation was performed, revealing that the combination of P53 and CA9 with the clinical factors including age and degree of dysplasia achieved the highest prediction accuracy. We constructed a nomogram with the identified prognostic factors for predicting the 5-, 10-, and 15-year progression free survival of OL.

CONCLUSIONS: The proposed nomogram may be useful for the accurate and individual prediction of the transformation to SCC in OL patients and may help clinicians offer appropriate treatments and follow up.

Summary statements

- This study aimed to develop a nomogram using previously identified markers found in progressive oral premalignant lesions.
- Patients were selected from a retrospective cohort of 160 patients, of which 22 patients had progressive lesions over a 3-year follow up period.
- Age, p53, and CA-9 were found to be significant variables in the nomogram that allowed for 0.85 prediction accuracy.

Strengths

- The prediction model is helpful in counseling patients with oral premalignant lesions. Since only 10-20% of these lesions progress to carcinoma, having a prediction model that allows patients to be risk-stratified is critical
- The authors had a large cohort of patients with a small percentage progressing to carcinoma to study their model accurately.

Weaknesses

- Smoking, alcohol and pre-existing oral conditions (e.g., oral lichen planus) were not included as this data was not available
- Biomarkers selected were from studies that did not use current technologies to determine expression differences in progressive lesions.
- Addition of degree of dysplasia into model increases prediction accuracy, which creates a selection bias.

back to top

Recent Progress in Therapeutic Treatments and Screening Strategies for the Prevention and Treatment of HPV-Associated Head and Neck Cancer

S. N. Whang, M. Filippova and P. Duerksen-Hughes

from ***Viruses****, September 2017*

The rise in human papillomavirus (HPV)-associated head and neck squamous cell carcinoma (HNSCC) has elicited significant interest in the role of high-risk HPV in tumorigenesis. Because patients with HPV-positive HNSCC have a better prognosis than their HPV-negative counterparts, current therapeutic strategies for HPV^+ HNSCC are considered to be overly aggressive, highlighting a need for customized treatment guidelines for this cohort. Additional issues include the unmet need for reliable screening strategies for HNSCC, as well as the ongoing assessment of the efficacy of prophylactic vaccines for the prevention of HPV infections in the head and neck. This review also outlines a number of emerging therapeutic vaccines, as well as targeted molecular-based therapies for HPV-associated head and neck cancers. Overall, the future for developing novel and effective therapeutic agents for HPV-associated head and neck tumors is promising; continued progress is critical in order to meet the challenges posed by this growing epidemic.

Summary statements

- 80% higher incidence in males than in females and a lower incidence in African Americans than in Caucasians (4% in AA *vs.* 34% in Caucasian)
- De-intensification of postoperative radiation after surgical resection of HPV-associated OPSCC is being evaluated with ECOG 3311 clinical trial.
- In most tobacco-related tumors, the *TP53* gene is mutated and inactive, while the *TP53* gene in HPV-associated tumors is wild-type and functionally intact, with the protein being degraded by the HPV oncoprotein E6.
- The Radiation Therapy Oncology Group study (RTOG 1016) and De-ESCALaTE phase III trials are comparing conventional cisplatin concurrently with radiotherapy to cetuximab with concomitant radiation in HPV-driven locally advanced oropharyngeal squamous cell carcinoma (SCC).
- HPV infection has not been identified in approximately 10%–20% of $p16^+$ HNSCC.
- RT-PCR has been proposed to detect the presence of E6/E7 mRNA and may be the gold standard for fresh tumor samples, since the expression of these two oncogenes is characteristic of functional HPV infection and cell transformation. However, this method requires further examination.
- HPV-16 E6 and E7 oncoproteins have become popular viral targets for therapeutic vaccines since they are consistently expressed in HPV malignancies and are critical in the transformation process.

Strengths

- An excellent comprehensive review of the current strategies for prevention and treatment of HPV associated OPSCC.
- This review provides a detailed insight into research on therapeutic vaccines, targeted therapies.

Weaknesses

- As this review was written in 2015, the 8th edition changes to the staging of HPV + OPSCC have not been alluded to in the review. Although not a weakness per se, a reference to this would have been helpful in the management section of the manuscript.

back to top

Kinetics of the Human Papillomavirus Type 16 E6 Antibody response prior to oropharyngeal cancer

Kreimer AR, Johansson M, Yanik EL, Katki HA, Check DP, Lang Kuhs KA, Willhauck-Fleckenstein M, Holzinger D, Hildesheim A, Pfeiffer R, Williams C, Freedman ND, Huang WY, Purdue MP, Michel A, Pawlita M, Brennan P, Waterboer T.

from ***Journal of the National Cancer Institute****, August, 2017*

Background: In a European cohort, it was previously reported that 35% of oropharyngeal cancer (OPC) patients were human papillomavirus type-16 (HPV16) seropositive up to 10 years before diagnosis vs 0.6% of cancer-free controls. Here, we describe the kinetics of HPV16-E6 antibodies prior to OPC diagnosis.

Methods: We used annual serial prediagnostic blood samples from the PLCO Cancer Screening Trial. Antibodies to HPV were initially assessed in prediagnostic blood drawn at study enrollment from 198 incident head and neck cancer patients (median years to cancer diagnosis = 6.6) and 924 matched control subjects using multiplex serology, and subsequently in serial samples (median = 5/individual). Available tumor samples were identified and tested for HPV16 RNA to define HPV-driven OPC.

Results: HPV16-E6 antibodies were present at baseline in 42.3% of 52 OPC patients and 0.5% of 924 control subjects. HPV16-E6 antibody levels were highly elevated and stable across serial blood samples for 21 OPC patients who were seropositive at baseline, as well as for one OPC patient who seroconverted closer to diagnosis. All five subjects with HPV16-driven OPC tumors were HPV16-E6-seropositive, and the four subjects with HPV16-negative OPC tumors were seronegative. The estimated 10-year cumulative risk of OPC was 6.2% (95% confidence interval [CI] = 1.8% to 21.5%) for HPV16-E6-seropositive men, 1.3% (95% CI = 0.1% to 15.3%) for HPV16-E6-seropositive women, and 0.04% (95% CI = 0.03% to 0.06%) among HPV16-E6-seronegative individuals.

Conclusions: Forty-two percent of subjects diagnosed with OPC between 1994 and 2009 in a US cohort were HPV16-E6 seropositive, with stable antibody levels during annual follow-up for up to 13 years prior to diagnosis. Tumor analysis indicated that the sensitivity and specificity of HPV16-E6 antibodies were exceptionally high in predicting HPV-driven OPC.

Summary statements

The HPV16-E6 biomarker has a high utility for predicting the cumulative long term risk for subsequent development of oropharyngeal cancer. All patients with subsequent development of oropharyngeal cancer and who were HPV16-E6 antibody positive at baseline continued to have consistently elevated HPV16-E6 levels during their follow up.

Strengths
This study used serial samples of HPV16-E6 antibody testing up to the diagnosis of oropharyngeal cancer over a long term follow up period, in contrast to previously published studies that have only utilized detection of antibodies at a single time point. The confirmation of HPV tumor status in oropharyngeal cancer patients was performed with the gold standard of HPV RNA analysis

Weaknesses
There were a limited number of oropharyngeal cancer patients overall in the study cohort. This is largely a reflection of oropharyngeal cancer being a rare cancer. While this study provides new clues on how to approach secondary prevention of HPV associated oropharyngeal cancer, it still does not adequately identify a high risk population for which screening for oropharyngeal cancer would be justified.

back to top

Erlotinib and the Risk of Oral Cancer: The Erlotinib Prevention of Oral Cancer (EPOC) Randomized Clinical Trial

William WN Jr, Papadimitrakopoulou V, Lee JJ, Mao L, Cohen EE, Lin HY, Gillenwater AM, Martin JW, Lingen MW, Boyle JO, Shin DM, Vigneswaran N, Shinn N, Heymach JV, Wistuba II, Tang X, Kim ES, Saintigny P, Blair EA, Meiller T, Gutkind JS, Myers J, El-Naggar A, Lippman SM.

*from **JAMA Oncology**, February 2016*

IMPORTANCE: Standard molecularly based strategies to predict and/or prevent oral cancer development in patients with oral premalignant lesions (OPLs) are lacking.

OBJECTIVE: To test if the epidermal growth factor receptor inhibitor erlotinib would reduce oral cancer development in patients with high-risk OPLs defined by specific loss of heterozygosity (LOH) profiles. Secondary objectives included prospective determination of LOH as a prognostic marker in OPLs.

DESIGN: The Erlotinib Prevention of Oral Cancer (EPOC) study was a randomized, placebo-controlled, double-bind trial. Accrual occurred from November 2006 through July 2012, with a median follow-up time of 35 months in an ambulatory care setting in 5 US academic referral institutions. Patients with OPLs were enrolled in the protocol, and each underwent LOH profiling (N = 379); they were classified as high-risk (LOH-positive) or low-risk (LOH-negative) patients based on their LOH profiles and oral cancer history. The randomized sample consisted of 150 LOH-positive patients.

INTERVENTIONS: Oral erlotinib treatment (150 mg/d) or placebo for 12 months.

MAIN OUTCOMES AND MEASURES: Oral cancer-free survival (CFS).

RESULTS: A total of 395 participants were classified with LOH profiles, and 254 were classified LOH positive. Of these, 150 (59%) were randomized, 75 each to the placebo and erlotinib groups. The 3-year CFS rates in placebo- and erlotinib-treated patients were 74% and 70%, respectively (hazard ratio [HR], 1.27; 95% CI, 0.68-2.38; P = .45). The 3-year CFS was significantly lower for LOH-positive compared with LOH-negative groups (74% vs 87%, HR, 2.19; 95% CI, 1.25-3.83; P = .01). Increased EGFR gene copy number correlated with LOH-positive status (P < .001) and lower CFS (P = .01). The EGFR gene copy number was not predictive of erlotinib efficacy. Erlotinib-induced skin rash was associated with improved CFS (P = .01).

CONCLUSIONS AND RELEVANCE: In this trial, LOH was validated as a marker of oral cancer risk and found to be associated with increased EGFR copy number (the target of the intervention). Erlotinib did not, however, improve CFS in high-risk patients with LOH-positive or high-EGFR-gene-copy-number OPLs. These results support incorporation of LOH testing as a prognostic tool in routine clinical practice but do not support erlotinib use in this setting.

Summary statements

- Large prospective randomized placebo-controlled clinical trial that evaluated the use of Erlotinib for prevention of oral cancer.
- There was no difference in oral cancer development between the patient cohorts receiving Erlotinib or placebo.
- Subjects who received Erlotinib and who developed a rash (> Grade 2) had better outcomes than those who did not develop a rash.

Strengths

- The primary end-point of the trial was development of cancer.
- Prospectively validated loss of heterozygosity (LOH) in oral mucosa has a biomarker of oral cancer risk.
- Identified high-EGFR-gene-copy-number as a potential marker of risk of oral cancer.

Weaknesses

- The patients enrolled in the trial were at very high risk for oral cancer development; these results may not also apply to people in the general population with oral premalignant lesions.
- LOH is a prognostic marker and may not be the most suited to identify whether EGFR was the best target in individuals with oral premalignant lesions.

back to top