



SESSION 4 - Viruses related cancers

1. DEVELOPMENT AND EXTERNAL VALIDATION OF NOMOGRAMS IN OROPHARYNGEAL CANCER PATIENTS WITH KNOWN HPV-DNA STATUS: A EUROPEAN MULTICENTRE STUDY (OROGRAMS)

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2. EVALUATION OF THE 8TH TNM CLASSIFICATION ON P16-POSITIVE OROPHARYNGEAL SQUAMOUS CELL CARCINOMAS IN THE NETHERLANDS, AND THE IMPORTANCE OF ADDITIONAL HPV DNA-TESTING

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3. DEFINING HPV-DRIVEN NEOPLASTIC TRANSFORMATION INFECTION IN OROPHARYNGEAL CARCINOMA. A MOVING TARGET IN HEAD AND NECK ONCOLOGY

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4. THE SUMO CONJUGATING ENZYME UBC9 IN HPV-RELATED HEAD AND NECK CANCER

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5. HIGH INFILTRATION OF CD68+ MACROPHAGES IS ASSOCIATED WITH A POOR PROGNOSIS OF HNSCC PATIENTS AND IS REDUCED BY HPV16-E6 ONCOPROTEIN

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6. HPV TESTING IN FINE-NEEDLE ASPIRATES OF CERVICAL LYMPH NODE METASTASIS FROM OROPHARYNGEAL SQUAMOUS CELL CARCINOMA

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7. CORRELATION BETWEEN HUMAN PAPILLOMA VIRUS (HPV) STATUS AND QUANTITATIVE MR PARAMETERS INCLUDING DIFFUSION-WEIGHTED IMAGING AND TEXTURE FEATURES IN OROPHARYNGEAL CARCINOMA

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8. HUMAN IMMUNODEFICIENCY VIRUS: ARE WE TESTING IN NEW HEAD AND NECK CANCER DIAGNOSES

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1. Development and external validation of nomograms in oropharyngeal cancer patients with known HPV-DNA status: a European multicentre study (OroGrams)

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Rationale: The proxy-marker for human papillomavirus (HPV), p16, is included in the new AJCC8th/UICC8th staging system, but due to incongruence between p16-status and HPV-infection, single biomarker evaluation could lead to misallocation of patients. We established nomograms for overall- (OS) and progression-free survival (PFS) in patients with oropharyngeal squamous cell carcinoma (OPSCC) and known HPV-DNA and p16-status, and validated the models in cohorts from high and low prevalent HPV-countries.

Materials and methods: Consecutive OPSCC patients treated in Denmark, 2000-2014 formed the development cohort. The validation cohorts were from Sweden, Germany, and the UK. We developed nomograms by applying a backward selection procedure for selection of variables, and assessed model performance.

Results: In the development cohort 1,313 patients, and in the validation cohorts 344 German, 503 Swedish, and 463 British patients, were included. For the OS nomogram, age, gender, combined HPV-DNA and p16-status, smoking, T-, N-, and M-status, and UICC-8 staging were selected, and for the PFS nomogram the same variables except UICC-8 staging. The nomograms performed well in discrimination and calibration.

Conclusions: Our nomograms are reliable prognostic methods in patients with OPSCC. Combining HPV-DNA and p16 is essential for correct prognostication. The nomograms are available at www.oroagrams.org.

2. Evaluation of the 8th TNM classification on p16-positive oropharyngeal squamous cell carcinomas in the Netherlands, and the importance of additional HPV DNA-testing

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Rationale: Oropharyngeal squamous cell carcinomas (OPSCCs) are traditionally caused by smoking and excessive alcohol consumption. However, in the last decades high-risk human papillomavirus (HR-HPV) infections play an increasingly important role in tumorigenesis. HPV-driven OPSCCs are known to have a more favorable prognosis, which has led to important and marked changes in the recently released TNM-8. In this edition, OPSCCs are divided based on p16-immunostaining, with p16-overexpression as surrogate marker for the presence of HPV. The aims of this study are to evaluate TNM-8 on a Dutch consecutive cohort of patients with p16-positive OPSCC and to determine the relevance of additional HPV DNA-testing.

Materials and methods: All OPSCC patients without distant metastases at diagnosis and treated with curative intent at VU University Medical Center (2000-2015) and Erasmus Medical Center (2000-2006) were included (N = 1,204). HPV-status was established by p16-immunostaining followed by HPV DNA-PCR on the p16-immunopositive cases. We compared TNM-7 and TNM-8 using the Harrell's C index.

Results: In total, 388 of 1,204 (32.2%) patients were p16-immunopositive. In these patients, TNM-8 had a markedly better predictive prognostic power than TNM-7 (Harrell's C index 0.63 versus 0.53). Of the 388 p16-positive OPSCCs, 48 tumors (12.4%) were HPV DNA-negative. This subgroup had distinct demographic, clinical and morphologic characteristics and showed a significantly worse five-year overall survival compared to the HPV DNA-positive tumors (P < 0.001).

Conclusions: TNM-8 has a better predictive prognostic power than TNM-7 in patients with p16-positive OPSCC. However, within p16-positive OPSCCs there is an HPV DNA-negative subgroup with distinct features and a worse overall survival, indicating the importance to perform additional HPV DNA-testing when predicting prognosis and particularly for selecting patients for de-intensified treatment regimens.

3. Defining HPV-driven neoplastic transformation infection in oropharyngeal carcinoma. A moving target in head and neck oncology

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Rationale: The definition of HPV status in oropharyngeal squamous cell carcinoma (OPSCC) is crucial for the choice of treatment. Viral oncogene E6 and E7 expression is considered evidence of HPV-driven neoplastic transformation, however mRNA assessment with RT-PCR is limited by its technical complexity, and algorithms pairing HPV DNA expression and p16 immunostain, as an indirect evidence of E6 oncogene activity, are currently applied in the diagnostic practice. Recently, an in situ test to detect viral oncogene the expression on histopathological slides has been proposed as a stand-alone test to classify HPV-associated OPSCC. Only limited data have been published on the correspondence between the two mRNA-based tests, and with other markers of HPV infection. We compared mRNA rt-PCR and ISH with HPV DNA and oncogene target expression in OPSCC to define their accuracy in detecting infections affecting oncogenic pathways.

Materials and methods: We collected fresh and formalin fixed samples from 54 consecutive OPSCC patients. Immunostains for p16, pRB, Cyclin D1, p53, HR HPV DNA (Inform HPV family 16 Ventana) and mRNA (RNA scope, ACD Bio) in situ hybridization (ISH), and SPF10 LiPA genotyping (Furejbio) were performed on fixed samples. Frozen samples were used for qRT-PCR amplification of HPV E6 mRNA.

Results: HR HPV mRNA qRT-PCR was positive in 31 samples (57.4%) and mRNA ISH in 24 (44.4%). Of the 7 mRNA PCR+/ISH- samples, 2 were positive for p16 and HPV16 DNA; 5 expressed HR HPV DNA (16 in 3 cases, 35 and 33 in the others) and were negative for p16 and DNA ISH. These 5 samples also expressed pRB. All the HR HPV DNA+ cases, including the 5 that were p16-, expressed viral mRNA by qRT-PCR; all the HR HPV DNA- cases, including 4 that were p16+, were negative for viral mRNA.

Conclusions: Studies comparing HPV identification methods with the gold standard of oncogene mRNA qRT-PCR are complicated by the presence of discordant cases. We confirmed the occurrence of p16 expression independent of viral oncogenesis. Our findings of mRNA qRT-PCR+/ISH- cases (16%) with no p16 stain/pRB loss challenges the assumption of a necessary correlation between HPV oncogene expression, pRB pathway inactivation, and more generally, HPV-driven neoplastic transformation, and suggest that the definition of HPV-driven oncogenesis in OPSCC at the molecular level still needs further investigation.

4. The SUMO conjugating enzyme UBC9 in HPV-related Head and Neck Cancer

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Rationale: We have recently demonstrated that Ubc9 protein (i) is up-regulated in HPV-positive HNC and cervical lesions and (ii) accumulates in E6/E7-expressing human keratinocytes due to autophagy impairment. Our current preliminary results are also indicating that we can recapitulate this phenotype in HNC cell lines, albeit the mechanisms resulting in Ubc9 up-regulation in HPV+ HNC seem diverse.

Materials and methods: Head and Neck Cancers Cell Lines and Primary Tissues.

Results: In both primary tissues and HNC cell lines Ubc9 is over-expressed in correlation with HPV positivity, in particular with HPVE6 oncoprotein expression, in agreement with our primary Human Keratinocytes (HKs) data. Concordantly, we observed a decrease in Ubc9 expression upon HPVE6/E7 silencing in HPV+ HNC cell lines. However, our preliminary data show that accumulation of Ubc9 is not only dependent on autophagy impairment. Other possible mechanisms will be discussed.

Conclusions: Our findings can lead to the finding of novel therapeutic targets and possible biomarkers of HPV status.

5. High Infiltration of CD68+ Macrophages Is Associated with a Poor Prognosis of HNSCC Patients and Is Reduced By HPV16-E6 Oncoprotein

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Rationale: HPV-related HNSCCs have seen their incidence increase over the last few decades. The reaction of the host immune system against these tumors remains biologically complex. Here, we investigated CD68+ macrophage number, reporting its prognostic value in comparison to other risk factors. We also examined CD68+ macrophage infiltration during disease progression regarding the impact of HPV infection and we studied the role of HPV16-E6/E7 oncoproteins in CD68+ macrophage recruitment.

Materials and methods: CD68+ macrophage number was evaluated in 10 cases of tumor-free peritumoral epithelia, 43 cases of low grade dysplasia, 45 cases of high grade dysplasia and 110 cases of carcinoma. Our in vivo model was developed on 80 C3H/HeN mice orthotopically injected with HPV16-E6, -E7 or -E6/E7-transfected SCC-VII cell line.

Results: We found that high CD68+ macrophage number in the intra-tumoral compartment is associated with shorter patient survival (RFS: $p=0.001$; OS: $p=0.01$). Multivariate analyses reported that CD68+ macrophage infiltration, as well as tumor stage, are strong and independent prognostic factors compared other risk factors. CD68+ macrophage number increases during HNSCC progression, both in intra-epithelial ($p<0.001$) and stromal compartments ($p<0.001$). A higher density of CD68+ macrophages was observed in advanced stages. Patients with transcriptionally active HPV infection have higher CD68+ macrophage density than HPV-negative ones ($p=0.003$). Finally, we found a higher infiltration of CD68+ macrophages in SCC-VII-E7+ and SCC-VII-E6/E7+ tumors than in SCC-VII-E6+ ones ($p=0.029$ and $p<0.001$) in mice.

Conclusions: The extent of CD68+ macrophage infiltration is a significant prognostic factor in HNSCC patients. The recruitment of macrophages increases during disease progression but is inhibited by HPV16-E6 oncoprotein.

6. HPV Testing in Fine-Needle Aspirates of cervical lymph node metastasis from Oropharyngeal Squamous Cell Carcinoma

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Rationale: The Human Papillomavirus (HPV) is involved in the pathogenesis of oropharyngeal squamous cell carcinoma (OPSCC), accounting for around 30% of the cases. In up to 50% of the cases, OPSCC patients present with a neck mass and a very small tumor. In a few cases, the fine needle aspirate (FNA) from cervical lymph nodes is the only specimen available for ancillary molecular analysis. We aimed to compare the results of HPV testing in FNA and corresponding formalin-fixed paraffin-embedded (FFPE) primary cancer tissue.

Materials and methods: Cervical lymph node FNA of patients with suspected head and neck neoplasm were collected in PreservCyt. Liquid based cytology was performed. Presence of HPV was analyzed by INNO-LiPA HPV genotyping Extra II on both cytological and FFPE samples.

Results: Thirty-seven FNA were collected. The diagnosis of carcinoma was reported in 26 cases (70.3%), 10 (27.0%) showed necrosis and were suspicious for cancer, while 1 (2.7%) was negative for carcinoma. This case was excluded from the analysis. For all the patients, the primary tumor was identified. For all the FNA specimens the corresponding OPSCC tissues were thus available. HPV was detected in 66.7% of both the cytological and histological samples, and 87.4% of them were positive for HPV16. The other HPVs detected were HPV18, HPV26 and HPV35. The comparison of HPV results for the 36 matched FNA and FFPE samples included in the analysis evidenced a complete concordance for HPV status and HPV type-specificity (Raw agreement=1; Cohen K=1).

It is worth noting that the 10 cases with a cytological diagnosis of necrosis, in which no representative cancer cells could be evidenced, showed a valid HPV result, with 6 HPV positive and 4 HPV negative cases, completely concordant with the corresponding FFPE tumor.

Conclusions: The HPV status of FNA specimens from metastatic cervical lymph nodes reflected that of the corresponding primary OPSCC, even when the FNA showed necrosis and was only suspicious for cancer. These cytological samples thus represent a useful tool to identify HPV-related OPSCC patients and provide prognostic information, indicating an improved survival for these patients. Additionally, in case of unknown primary tumor HPV-positivity on the FNA suggests an oropharyngeal origin and may thus help avoid unnecessary treatments of other head and neck sites.

7. Correlation between Human Papilloma Virus (HPV) status and quantitative MR parameters including diffusion-weighted imaging and texture features in oropharyngeal carcinoma

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Rationale: This study is aimed to investigate the association of quantitative MR features including diffusion-weighted imaging (DWI) and Human Papilloma Virus (HPV) status in oropharyngeal squamous cell carcinoma (OPSCC).

Materials and methods: We retrospectively analyzed MR studies of 59 T2-T4 OPSCC. HPV status was determined by viral DNA detection on tissue samples. MR protocol included T2-weighted, contrast-enhanced T1-weighted (VIBE) and DWI sequences. Parametric maps of Apparent Diffusion Coefficient (ADC) were obtained from DWI sequences. Texture analysis was performed on T2 and VIBE sequences and on ADC maps. Differences in quantitative MR features between HPV-positive and HPVnegative tumors and among subgroups of patients stratified by smoking status were tested using non-parametric Mann-Whitney test; false discovery rate was controlled using Benjamini-Hochberg correction; a predictive model for HPV status was built using multivariate logistic regression.

Results: Twenty-eight patients had HPV-positive while 31 patients had HPV-negative OPSCC. HPV-positive tumors had a significantly lower mean ADC compared to HPV-negative tumors (median 850,87 vs median 1033,68; pvalue <0.0001). Texture features had a lower discriminatory power for HPV status. Skewness on VIBE sequences was significantly higher in the subgroup of HPVpositive and smoker patients (p=0.003). A predictive model based on smoking status and mean ADC yielded to sensitivity of 83.3% and specificity 92.6% in classifying HPV status.

Conclusions: ADC is significantly lower in HPV-positive compared to HPVnegative OPSCC. ADC and smoking status allowed noninvasive prediction of HPV status with a good accuracy. These results should be validated and further investigated on larger prospective studies.

8. Human Immunodeficiency Virus: Are We Testing in New Head and Neck Cancer Diagnoses

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Rationale: Head and neck cancer is one of the most common types of cancer seen in patients with Human Immunodeficiency Virus (HIV). As a result of this the British HIV Association (BHIVA) guidelines state that all patients with new diagnoses of head and neck cancer should be offered a HIV test. To assess how many Head and Neck departments' offer HIV tests routinely in newly diagnosed head and neck cancer patients.

Materials and methods: All head and Neck multidisciplinary teams (MDT) were identified in England. Head and Neck Specialist Nurses from these MDT's were sent a questionnaire asking whether their department routinely offers HIV tests in new head and neck cancer patients.

Results: We identified 49 Head and Neck cancer MDT's and all Clinical Nurse Specialists associated with these MDT's were asked to complete the questionnaire. We found that only one of the respondents to the questionnaire routinely offered HIV testing in this high-risk patient group. Many of the respondents were not aware of the BHIVA guidelines and how they were related to Head and Neck cancers.

Conclusions: Head and Neck cancer patients should be offered HIV testing. This observational study highlights that as a specialty we are not aware of, and consequently, not complying with routine HIV testing.