PROGNOSTIC FACTORS IN HPV-POSITIVE OROPHARYNGEAL CARCINOMA
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Background:

Human papillomavirus (HPV) is implicated as the causative factor in most (>75%) oropharyngeal carcinoma (OPSCC) patients. The majority (85%) of patients with HPV-positive OPSCC have improved survival compared to those with HPV-negative disease, leading to treatment “de-escalation” trials in order to reduce treatment-related morbidity. However, identifying the significant minority (15%) that will have a poor outcome, despite being HPV-positive has been a challenge. Both heavy smoking and advanced nodal disease can alter outcome in these patients, but there is no consensus opinion on how to best identify the minority of HPV-positive patients with poor survival. The aim of this study was to compare possible molecular and clinical prognostic markers, in HPV-positive OPSCC.

Methods:

We performed a retrospective case note analysis of 274 patients treated consecutively for OPSCC at University Hospital Southampton (UHS) and Poole Hospital Foundation Trust (PFT) between 2000 and 2010. HPV-status was determined using p16 immunohistochemistry and HPV in-situ hybridisation. Only tumours that were positive for both were considered HPV-positive. The effect of clinical features (overall TNM stage, T-stage, N-stage, smoking status) and molecular markers (p53 and EGFR expression, tumour infiltrating lymphocytes (TILs, graded as low, moderate or high)) on disease specific survival (DSS) was assessed using a combination of Kaplan Meier analysis and both univariate and multivariate Cox regression. Likelihood ratios for 3-year mortality were calculated to assess the strength of each marker as a prognostic factor. Logistic regression was used to develop a predictive model for 3-year mortality in patients treated at UHS. This model was validated in patients treated at PFT.

Results:

Of the 274 patients, 149 (54%) were classified as HPV-positive. HPV-positive tumours had improved survival compared to HPV-negative tumours (log rank p<0.001, adjusted HR 0.45, p=0.005). HPV-positive tumours were subsequently analysed further. On Kaplan Meier analysis, reduced survival was associated with advanced T-stage (T3/4, p=0.001), advanced N-stage (N2b-N3, p=0.05), heavy smoking (>10 pack years, p<0.001), and a low number of TILs (p=0.002). Overall TNM stage, p53 and EGFR expression did not significantly predicted for survival. On univariate Cox regression, heavy-smoking (HR 5.81), low TIL-levels (HR 5.67), advanced T-stage (HR 3.42) and advanced N-stage (HR 2.29) were negative prognostic markers. On multivariate analysis (adjusted for age, treatment and all significant univariate markers) only low TIL-levels (HR 5.97, p=0.009) and heavy-smoking (HR 4.98, p=0.001) were significant predictors of DSS. Interestingly, patients with tumours that were HPV-positive but with low TIL-levels had no difference in survival from those with HPV-negative tumours (HR 1.01, p=0.98). The best prognostic marker for 3-year mortality was low TIL-levels (Likelihood Ratio [LR] 3.30). A predictive model was developed containing T-stage, smoking status and TIL-levels. The LR for this model in patients treated at PFT was 11.9.

Conclusions:
These results suggest that HPV-positive patients who have low TIL-levels, or are heavy-smokers, are at increased risk of disease specific death. We have developed a prognostic model which is highly predictive of 3-year mortality. We suggest that patients who are classified as "high risk" by this model should not be included in de-escalation trials.
DETECTION OF HPV-ASSOCIATED OROPHARYNGEAL TUMOURS IN A 16-YEAR COHORT: MORE THAN MEETS THE EYE

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Purpose HPV-associated oropharyngeal squamous cell carcinomas (OpSCC) have been reported at high frequencies in certain countries and are considered a separate entity because OpSCC are consistently associated with an improved disease-free and overall survival. Recently, an algorithm has become available that reliably detects clinically relevant HPV in tumour tissue. However no complete cohorts have been tested. The aim of this study was to determine the prevalence of active high-risk HPV infection in a complete cohort of tonsillar and base-of-tongue squamous cell carcinomas from the Northern Netherlands collected over a 16-year period.

Material and Methods Based on the Dutch cancer registration, we collected clinical, pathological, and follow-up data and formalin-fixed paraffin embedded samples of a complete cohort of 196 tonsillar and base-of-tongue oropharyngeal squamous cell carcinomas treated in our centre from 1997-2012, as well as a random selection of 200 oral squamous cell carcinomas (OSCC) treated from 1997-2008. HPV testing was performed using an established algorithm of p16 immunohistochemistry, followed by HPV-PCR of the p16-positive cases. HPV-BRISH was performed as extra control of p16 negative cases.

Results p16 staining could be assessed on 193 OpSCC. 64/193 (33%) were p16+. Of these 64 cases 47 (73%) were HPV-GP+, of which 42 cases were HPV16+, 1 HPV18+, 3 HPV33+ and 1 HPV35+. HPV-associated tumour proportion increased from 13% between 1997-2004, to 30% between 2005-2012. HPV-positivity was an independent predictor for longer disease-specific survival (HR=0.22; 95%CI:0.10-0.47), and loco-regional disease-free survival (HR=0.10; 95%CI:0.02-0.42). Only one OSCC was HPV+.

Conclusions The incidence of HPV-associated OpSCC in our cohort is low but increasing rapidly. The strict detection algorithm, analysis of disease-specific survival, and the complete cohort which included also palliatively treated patients, may influence the reported prevalence and prognostic values of HPV in OpSCC.
HUMAN PAPILLOMAVIRUS POSITIVE STATUS AND/OR P16INK4A OVEREXPRESSION VERSUS HPV NEGATIVE STATUS AND/OR P16INK4A NON-OVEREXPRESSION FOR THE PROGNOSIS OF HEAD AND NECK SQUAMOUS CELL CARCINOMA

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Background: Each year, 600,000 new cases of head and neck squamous cell carcinoma (HNSCC) are diagnosed worldwide. Common risk factors for most forms of HNSCC include heavy consumption of tobacco and/or alcohol, although the oropharyngeal variant (OPSCC) is less likely to be associated with tobacco and alcohol exposure and more often correlated with human papillomavirus (HPV) infection. Head and neck squamous cell carcinoma (HNSCC) patients with human papillomavirus (HPV) infection have better prognosis than HNSCC patients who are HPV negative. Although p16INK4a expression is used as a surrogate marker for HPV infection, there is controversy as to whether p16INK4a reliably indicates HPV infection. Our aims were to evaluate the prognostic value of HPV DNA presence and p16INK4a expression for HNSCC survival.

Methods: Prospectively collected HNSCC samples (n=150) were analyzed for HPV DNA, E6/E7 mRNA, and p16INK4a expression by polymerase chain reaction and immunohistochemistry. P16INK4a expression level was scored from 0 to 4 according to the percentage of p16INK4a-positive cells, with overexpression defined as > 40% positive cells.

Results: Ten tumors were nasopharyngeal, 53 oropharyngeal, 39 hypopharyngeal, 24 laryngeal, and 24 were located in the oral cavity. Presence of HPV DNA was observed in 47 (31.3%) of HNSCC samples, but only 21 also exhibited HPV mRNA expression. Inter-rater agreement was low between p16INK4a expression and HPV DNA presence and between p16INK4a expression and HPV mRNA expression, but was good between p16INK4a overexpression and HPV mRNA expression (κ = 0.69). Five-year recurrence-free survival was significantly higher for HPV DNA-positive patients than for HPV DNA-negative patients (93.4% vs. 68.4%; P = 0.003), for HPV mRNA-positive patients than for HPV mRNA-negative patients (100% vs. 72.0%; P = 0.014), and for patients overexpressing p16INK4a (93.1% vs. 71.8% of patients with < 40% positive cells; P = 0.031). Patients with HPV DNA-negative HNSCC not overexpressing p16INK4a had significantly lower recurrence-free survival than all other HNSCC patients (P = 0.003). Multivariate analysis revealed that HPV DNA-negative status and T stage 4 predicted significantly lower recurrence-free survival, while patients overexpressing p16INK4a showed significantly improved recurrence-free survival.

Conclusions: Overexpression of p16INK4a is a more accurate marker for active HPV infection in HNSCC than general p16INK4a-positive status. Patients overexpressing p16INK4a had good prognosis even if HPV negative. The combined evaluation of p16INK4a expression and HPV DNA status may improve prognostic accuracy in HNSCC.
PIK3CA MUTATIONS OCCUR MORE FREQUENTLY IN HUMAN PAPILLOMAVIRUS-CONTAINING OROPHARYNGEAL CARCINOMAS AND ARE ASSOCIATED WITH POOR OVERALL SURVIVAL.

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Objective. The PI3K pathway and PIK3CA gene are frequently mutated in head and neck squamous cell carcinomas (HNSCC). Recent publications suggest that PIK3CA mutations occur more common in human papillomavirus (HPV)-positive HNSCC than in their HPV-negative counterpart. The aim of this study was to confirm this finding and correlate results with clinical data.

Material and method. DNA from all available oropharyngeal squamous cell carcinomas (OSCC) in the Maastricht University Medical Center from 2007/2008 (n=66) was used. Clinical 5-year follow-up data of all patients were collected. HPV status was determined by combining p16 expression with HPV-DNA-specific PCR analysis. PIK3CA mutation status was tested using high resolution melting (HRM) and pyrosequencing analysis.

Results. 64 out of 66 OSCC DNAs were eligible for PCR analysis, of which 26 were HPV-positive (41%). 5 out of 26 (19%) HPV-positive samples contained a PIK3CA mutation compared to one out of 38 (3%) HPV-negative samples (p=0.036). Interestingly, the HPV-positive group containing a PIK3CA mutation had a significantly worse 5-year overall survival (p=0.027). However, when taking into account disease specific survival and curative treatment intention, this correlation did not reach significance anymore. Furthermore, no association was found between TNM status and PIK3CA mutation status.

Conclusion. HPV-positive OSCC have a higher frequency of PIK3CA mutations than their HPV-negative counterparts. Besides the fact that PIK3CA mutations are associated with poor overall survival in HPV-positive tumors, they may also serve as predictive biomarkers for treatment with new PI3K pathway inhibitors. These findings should be further investigated in a larger patient group.
TREATMENT FOR RECURRENT/METASTATIC HPV+ HEAD & NECK CANCER BY MTOR INHIBITION

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Metastatic HPV+ HNSCC currently has limited effective treatment options. Lack of a metastatic model of HPV+ HNSCC closely recapitulating progression in humans has limited the ability to study and advance treatment of this fatal disease stage. To aid in treatment development we have recently described a HPV+ recurrent metastatic model in immune competent mice and begun to explore novel effective treatments. In the model, individual metastatic clones were characterized and, although none of the clones are identical, they were all found to be resistant to standard of care cisplatin/radiation therapy (CRT), to be more aggressive in growth in vivo, and to remetastasize to the lymph nodes/lungs at higher rates than their parental cell line, closely mimicking human disease.

While the metastatic lines all have significant differences in gene expression profiles, a common feature was overexpression of mTOR and genes associated with this signaling cascade. To then investigate whether mTOR inhibition would be an effective treatment for recurrent metastatic disease we tested treatment response both in vitro and in vivo. Lung metastasizing cell lines showed similar to elevated levels mTOR signaling by Western blot and protein array, and were inhibited in proliferation by low dose rapamycin in a dose dependent manner. Rapamycin enhanced cisplatin and radiation induced cytotoxicity to HNSCC cells in colony forming assays. In addition, rapamycin re-sensitized resistant lung metastasizing cell lines to treatment in vivo, significantly prolonging survival, improving long-term cures, and limiting lymph node and lung metastasis. Studies in immunocompromised mice showed that the ability of rapamycin to limit metastasis is independent of the anti-tumor immune response. We are currently evaluating mRNA and protein arrays to better understand the mechanism of rapamycin enhanced clearance and limited metastasis. This study implicates mTOR activation in HPV+ HNSCC recurrent/metastatic disease. Furthermore, inhibition of mTOR may not only enhance treatment of resistant, metastatic cell populations at the primary site, but also enhance systemic platinum based chemotherapy and limit distant metastasis.
ASSOCIATION OF TAP1 GENE POLYMORPHISM IN HUMAN PAPILLOMA VIRUS RELATED OROPHARYNGEAL CANCER IN NORTH INDIAN POPULATION.

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BACKGROUND: The TAP-1 gene product is involved in the processing of endogenous peptides that bind to MHC class I molecules. Mutations and/or polymorphism within this gene could alter the efficacy of the immune response which might be relevant for the development of cancer.

AIMS: To study the association of TAP1 gene polymorphism with genetic susceptibility to HPV related oropharyngeal cancer in North Indian population.

METHODS & OBSERVATIONS: This study consisted of 100 cases and 100 controls divided into three groups; Group A- HPV positive oropharyngeal cancer (17 patients), Group B- HPV negative oropharyngeal cancer (83 patients) and Group C as controls. DNA was isolated from peripheral blood sample of all controls and from tumor tissue in cancer patients and TAP1i333v polymorphism was determined by PCR-RFLP. Val/Val genotype was higher in control population. No significant difference in distribution of Val/Val genotype in Group A compared to Group C was found (OR=0.663, 95% CI=0.164-2.688, p=0.563) and Val/Val genotype was significantly decreased in Group B as compared to controls (OR=2.604, 95% CI= 1.030-6.566, p=0.039). No significant difference in distribution of Val/Val genotype in Group A compared to group B was found (OR=1.172, 95% CI=0.388-7.692, p=0.470). No significant difference was found in distribution of Val allele among Group A compared to controls (OR=0.665, 95% CI=0.302-1.468, p=0.311). Val allele was significantly decreased in the Group B compared to controls (OR=1.683, 95% CI=1.078-2.627, p=0.021). Val allele was significantly higher in controls than in cases (OR=1.650, 95% CI=1.083-2.515, p=0.019).

CONCLUSION: No association was identified between risk of HPV positive oropharyngeal cancer and TAP1i333v gene polymorphism. Individuals within Control group who were carriers of increased frequency of Val/Val genotype were protected from oropharyngeal cancer. Incidence of HPV positivity for oropharyngeal cancer was 17% in north Indian population.
BACKGROUND:

Although previous studies have investigated TP53 mutation in head and neck squamous cell cancers (HNSCC) as a mixed anatomical group, no study has investigated this in a homogenous set of well-annotated primary oropharyngeal squamous cell carcinomas (OPSCC). Many OPSCCs are caused by human papillomavirus (HPV), which acts to suppress wild type TP53. In those that are HPV negative, little is known about the TP53 status with regard to frequency and type of mutation present. This study addresses this gap in current knowledge. In this study we assess HPV positivity (p16 and chromogenic in situ hybridisation (CISH) for HPV DNA) and examine the correlation between the levels of TP53 by immunohistochemical (IHC) staining and mutation sequencing. It is important to note that not all TP53 mutations are equal, and there is variability in the TP53 protein structure, stability and DNA binding properties.

METHODS:

DNA was extracted from a cohort of 200 formalin fixed paraffin embedded primary (pre-radiotherapy) oropharyngeal tumours, for which there is detailed clinical annotation, facilitating correlation of TP53 mutation data with risk factors and outcome measures. The HPV status of these tumours was determined by IHC for p16, a protein used as a surrogate marker for HPV, and CISH for HPV DNA. DNA was extracted for Sanger sequencing, to detect mutations in the TP53 tumour suppressor gene within the DNA binding domain, where mutations most frequently occur. Correlation of clinical information and laboratory findings was carried out using appropriate statistical methodology.

RESULTS:

There was strong correlation between p16 and HPV DNA CISH for detection of HPV positivity. TP53 mutations were much more frequent in the HPV negative population. In a subset of 120 tumours, only one tumour was strongly positive on IHC staining for TP53, HPV DNA and p16. It was noted that tumours that were strongly positive IHC for TP53 correlated with poorer survival, whereas p16/CISH positivity correlated strongly with increased survival (p<0.01). There was also a correlation between TP53 mutation detection and strong TP53 IHC positivity, and the reliability of IHC in detecting mutations will be discussed. Details of TP53 mutation type and frequency will be presented for this cohort, with the most frequent mutation present at exon 5. TP53 mutation was inversely correlated with HPV positivity, but positively correlated with smoking history.

CONCLUSIONS:

This is the first study of its kind to look at both HPV and TP53 mutations in a large, well annotated OPSCC cohort, and preliminary results indicate that HPV positivity and TP53 mutation are almost always mutually exclusive events. Mutations of TP53 were located in exons 4 to 9, as is the case for other
cancers. The types of mutation will be discussed in detail. This has therapeutic relevance in the context of therapies targeted towards the TP53 gene, which can stabilise TP53 and induce senescence in cancer cells. In addition, there will be a need for stratification of tumours based on TP53 mutations, and this study provides a comprehensive account of this topic in a well-defined cohort.
DNA DAMAGE IN PERIPHERAL BLOOD CELLS PREDICTS OROPHARYNGEAL CANCER RISK
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Background: The levels of DNA damage caused by specific genotoxics are modulated by genetic and epigenetic factors, as well as life-style choices and are expected to be a major determinant of the individual susceptibility to cancer. Existing assays to detect DNA damage are quite limited in the types of DNA damage they can detect, and are not practical for population-based screenings. We developed a novel technique named primer-anchored DNA damage detection assay (PADDA) that reliably quantifies nucleotide and strand specific DNA damage in vivo. PADDA measures a wide-range of DNA damage lesions, has higher sensitivity than other available assays and is practical for population-based screening.

Aims: (1) To determine whether the levels of persistent DNA damage in oropharyngeal cancer patients differ from a control cohort. (2) To determine the DNA damage threshold that can optimally distinguish between cancer patients and controls. (3) To correlate the levels of DNA damage with known risk factors such as tobacco-smoking habits.

Methods: PADDA was used on a real-time PCR setting to quantify in vivo DNA damage in the p53 of the peripheral blood nucleated cells from 50 patients with oropharyngeal cancer and 50 non-cancer controls. To determine the damage threshold that could optimally distinguish between patients and controls, we constructed a receiver operating characteristic curve (ROC). Linear regression models were used to determine whether in vivo persistent DNA damage is associated with known risk factors for head and neck cancer.

Results: Our data show that oropharyngeal cancer patients have very high levels of DNA damage in both transcribed and non-transcribed strands of the p53 in peripheral blood cells at time of diagnosis. This damage is significantly higher than in non-cancer individuals (p<0.001). The ROC curve showed that DNA damage is an excellent diagnostic test with an accuracy of 93%. In addition, we were able to use the ROC curve to define the DNA damage threshold that optimally distinguishes between oropharyngeal cancer patients and non-cancer controls with 88% sensitivity and 84% specificity. A possible correlation between the in vivo levels of DNA damage and individual parameters is currently being analyzed.

Conclusion: Our study shows for the first time that oropharyngeal cancer patients have very high levels of DNA damage in peripheral blood cells at time of diagnosis. Of major clinical importance, our study documents that measuring DNA damage in peripheral blood cells has a high potential to assess the risk of oropharyngeal cancer. PADDA is a sensitive and affordable assay for the routine evaluation of DNA damage and repair with an unprecedented ability to quantify strand specific DNA damage. PADDA may become a critical tool to assess cancer risk and guide prevention strategies.

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DO EGFR EXPRESSION AND GENE COPY NUMBER INFLUENCE OUTCOME IN SURGICALLY TREATED OROPHARYNGEAL CANCER (OPSCC)?

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Background: The incidence of oropharyngeal cancer, particularly human papillomavirus (HPV) related OPSCC is on the rise in the United States and Europe. Epidermal growth factor receptor (EGFR) has been shown to be over expressed in head and neck squamous cell carcinoma, but there are conflicting reports of whether EGFR expression and or gene amplification influence survival in patients. We performed this study to evaluate the effects of EGFR expression and gene amplification in surgically treated OPSCC patients at the Ohio State University James Cancer Hospital.

Methodology: Tissue microarrays were built from paraffin embedded tumors of surgically treated patients with OPSCC. The TMA's were stained for EGFR expression using immunohistochemistry (IHC). EGFR gene amplification was assessed by fluorescent in-situ hybridization (FISH). EGFR stain proportion was scored on a linear scale. Stain intensity was scored as 1(none), 2(low), 3(moderate) and 4(high). Stain intensity and stain proportion were multiplied to obtain a quick score for each tumor. For the purpose of analysis, EGFR positive status was defined as following: EGFR stain proportion average >50%; stain intensity average >2, EGFR quick score average >200. EGFR amplification was scored as 1 (2 copies), 2 (>2 copies and 2-3 copies of centromere, polysomy) or 3 (>=4 copies, centromere not increased). Chromogenic in-situ hybridization was used to determine HPV16 positivity.

Results: In 223 patients, the 5 year overall survival (OS) was 59.25%. Ninety nine patients (44.59%) had HPV16 negative tumors and 123 (55.41%) had HPV positive tumors (n=1 missing). EGFR protein expression was not significantly associated with OS in patients of this surgically treated cohort (EGFR stain proportion p=0.9034; stain intensity 0.3771; quick score p=0.2031). However, EGFR expression was found to be inversely correlated with HPV16 status (EGFR stain proportion p=0.0404; stain intensity p=0.0673; quick score p=0.0245). Tumors from 127 patients (57.21%) had normal disomy (EGFR FISH score 1), 87 (39.19%) had polysomy (EGFR FISH score 2); 8 patients had an amplification (EGFR FISH score 3). EGFR expression was found to be directly correlated with EGFR FISH score 2 & 3 (EGFR stain proportion/EGFR FISH p<.001; stain intensity/EGFR FISH p= 0.004; quick score/EGFR FISH p=0.018).

EGFR gene amplification was significantly associated with OS (p<0.0001). Patients whose tumors had a FISH score of 3 had the poorest survival as compared to patients with EGFR FISH score 1 or 2. EGFR amplification or polysomy was also associated with poor overall survival when comparing EGFR FISH score of 1 versus 2 & 3 combined (p=0.0352). EGFR amplification and/or polysomy was found to be inversely associated with HPV status (p=0.0069). In addition, patients who smoked more than 10 pack-years had greater odds of having an EGFR amplification score of 2 or 3 (combined) as compared to those who smoked 10 pack-years or less (48% vs. 28%; p=0.009).

Conclusions: EGFR gene copy number abnormalities were significantly associated with poor survival. EGFR alterations (overexpression and high gene copy number) were inversely associated with HPV status in patients with OPSCC. EGFR pathway defects seem to play a greater role in HPV negative OPSCC.