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Article

Prognostic role of p16 overexpression in sinonasal squamous cell carcinoma: a retrospective analysis of Alberta patients

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Simple Summary: Sinonasal squamous cell carcinoma is rare amongst the general population and presents with historically poor prognosis. As a surrogate marker for human papillomavirus, p16 has been previously associated with improved prognosis and survival in squamous cell carcinoma of the oropharynx. In this study, we aim to establish correlation of p16 status with demographics, staging, treatment, and survival amonst a population-based sample comprised of patients from the Northern Alberta Head and Neck Tumour Board, Alberta Cancer Registry, and Alberta Cancer Research Biobank. Through improved understanding of pathologic factors implicated in sinonasal cancer, outcomes and prognosis can be better characterized and studied.

Abstract: Background: Sinonasal squamous cell carcinoma (SNSCC) is rare in the general population. No clear and consistent etiologic correlation between human papillomavirus and SNSCC has yet been delineated in literature. p16 is a tumour suppressor protein used as a surrogate marker for HPV. This study aims to evaluate the relationship between p16 overexpression in SNSCC and its role in prognosis and survival. Methods: A population-based retrospective analysis was performed using prospectively collected data from the Northern Alberta Head and Neck Tumour Board, Alberta Cancer Registry, and Alberta Cancer Research Biobank. p16 overexpression was analyzed from pathologic sample of patients meeting study criteria, and participants were dichotomized by status. Subsequently, nonparametric analysis of demographics, initial staging, and initial treatment were performed, and a Kapan-Meier curve was developed to assess differences in survival. Results: 16 patients were included in analysis. p16 overexpression was seen in 68.8% of patients. p16 positive and negative groups were comparable for age, gender, smoking status, stage, and treatment. A statistically significant five-year survival advantage was observed in patients with p16 positive SNSCC ($p = 0.013$). Conclusions: This is the first Canadian study to demonstrate a high prevalence of p16 positivity in SNSCC and its presence denoting a statistically significant survival advantage. Results demonstrate a previously unconfirmed role of oncogenic HPV in SNSCC.

Keywords: Sinonasal squamous cell carcinoma; Head and neck; HPV; p16; Survival

1. Introduction

Squamous cell carcinoma of the nasal and paranasal sinuses is rare in the general population, with an incidence rate of 0.5-1 per 100,000 [1,2]. While sinonasal squamous cell carcinomas (SNSCC) account for only 3% of malignant tumors in the head and neck, there remains no clear etiologic correlation between human papillomavirus (HPV) and SNSCC [3]. Treatment of SNSCC often includes primary surgical resection followed by

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adjuvant radiation therapy [4-6]. Despite treatment, prognosis for SNSCC is poor, with a reported 5-year survival rate of 40% [5,6].

While HPV has many strains, the most prevalent oncogenic strains are HPV-16 and HPV-18 [7]. Either HPV-16 or HPV-18 have been identified in nearly 99% of cervical cancers, and viral load of oncogenic strains is a previously identified risk factor for anal and oropharyngeal squamous cell carcinomas (OPSCC) [7-10]. HPV-positive OPSCCs occur in younger patients and possess distinct molecular features associated with improved treatment response and overall prognosis [11-16].

Overexpression of may be used as a surrogate marker for high-risk HPV infection, as documented for cervical and oropharyngeal SCCs [17-19]. p16 (p16INK4a) is a tumour suppressor protein, normally repressed by the retinoblastoma protein [18-21]. However, in tumours with transcriptionally active HPV, retinoblastoma protein is inactivated, resulting in p16 overexpression [8,22,23]. As a result, p16 overexpression has been extensively studied as a valuable marker for oncogenic HPV infection and can be detected using immunohistochemistry.

While previous literature has reported a greater disease-free survival in HPV associated SNSCC [24,25], there is a scarcity of evidence on the association of oncogenic HPV carrier status and sinonasal squamous cell carcinoma [26]. Bishop et al. (2013) reported p16 overexpression to be an appropriate biomarker for HPV presence, in SNSCC [27].

The current study aims to examine mortality associated with p16 overexpression in SNSCC among an Alberta population-based sample. It is hypothesized that oncogenic p16 carrier status will be associated with improved prognosis, as derived from literature surrounding similar head and neck malignancies.

2. Materials and Methods

Prospectively collected population-based data was used to conduct a retrospective analysis of p16 expression in sinonasal squamous cell carcinoma tumors. Ethical approval was obtained from the Health Research Ethics Board of the Alberta Cancer Committee (Approval: HREBA.CC-17-0431_REN1).

In the interest of comprehensively evaluating all eligible subjects in the province of Alberta, all anonymized patient data from the Northern Alberta Head and Neck Tumour Board (NAHNTB), Alberta Cancer Registry, and Alberta Cancer Research Biobank between 2008 – 2017 was examined. The NAHNTB is a multidisciplinary review board of patients with Head and Neck Cancer being treated in Northern Alberta. The Alberta Cancer Registry is a legislatively mandated and comprehensive population-based registry which records and maintains data on all cancer cases and deaths occurring in the province since 1942. The Registry records type of cancer and demographic patient information. Cancer-related deaths are recorded by the Registry using information from Alberta Vital Statistics and Statistics Canada. The Alberta Cancer Registry is operated by the Alberta Health Services – Cancer Care and is mandated by the Regional Health Authorities Act of Alberta [28]. The Alberta Cancer Research Biobank collects and stores various tumour samples from cancer patients across Alberta.

From the NAHNTB database, patients with available pathology reports were reviewed against inclusion and exclusion criteria (Table 1). Sixteen patients meeting inclusion criteria were identified and pathological evaluation was retrieved. Two researchers cross-referenced patients reports who met initial inclusion criteria, to ensure agreement of tumour origin from the sinonasal cavity and appropriate inclusion in this investigation. If there was uncertainty regarding origin of the tumour, diagnostic imaging studies were reviewed and correlated with pathology reports to determine inclusion.

Subsequently, participants were dichotomized by p16 expression status, either staining positively for overexpression, or negatively. p16 staining was considered positive when greater than 75% of the histologic sample has high-intensity, diffuse staining. p16 expression was based on third party initial pathological evaluation [29]. A Mann-Whitney

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U statistical analysis was employed for nonparametric evaluation of age, gender, smoking status, stage and treatment in the study population. A Kaplan-Meier curve was used to assess survival between the two groups. A p-value <0.05 was considered significant.

Table 1. Inclusion and exclusion criteria.

Inclusion Criteria	Exclusion Criteria
• >18 years of age • Biopsy proven sinonasal squamous cell carcinoma • Available p16 staining • Registration in available datasets (NAHNTB, Alberta Cancer Registry, or Alberta Cancer Research Biobank between 2008-2017)	• < 18 years of age • Non-sinonasal malignancy • No available p16 staining

3. Results

3.1. Participant Selection

During database search for patient selection, 35 patients were originally identified with SNSCC.

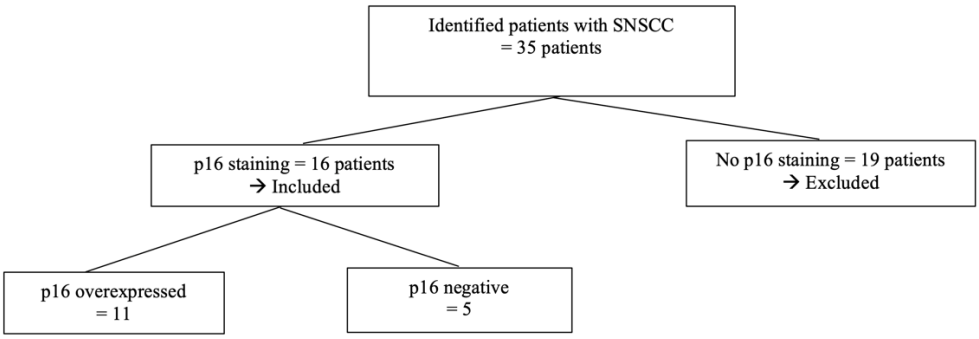

Figure 1. Patient review and study inclusion schematic.

3.2. Demographic Analysis

The p16 positive and negative groups were analyzed for age, gender, smoking status, stage at the time of biopsy, and initial treatment. Both groups were comparable for age, gender, smoking status, stage, and treatment (p=0.91, p=0.46, p=0.95, p=0.32 and p=0.17, respectively) (see Appendix 1, Table 2).

3.3. Survival Analysis

All-cause mortality between the p16 positive and negative groups over 5 years was examined. Analysis of 5-year survival revealed a statistically significant increased survival rate for p16-overexpressing SNSCC as compared to p16 negative tumours (p=0.013). (Figure 2).

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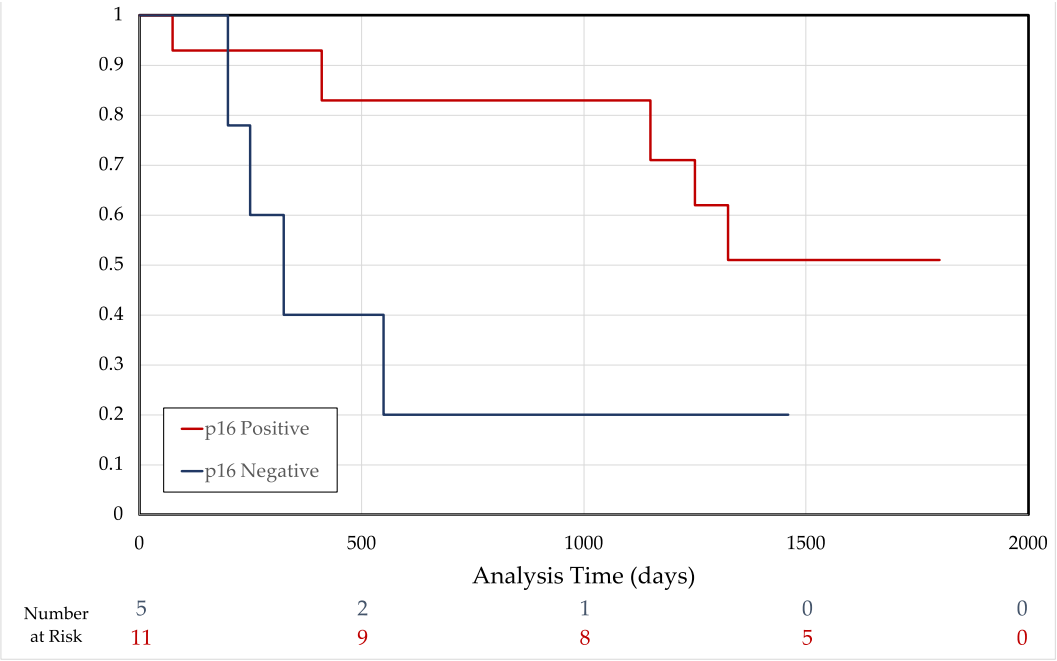

Figure 2. Kaplan-Meier survival curve of 5-year survival analysis.

4. Discussion

The data demonstrates a statistically significant 5-year survival advantage in patients diagnosed with p16 overexpressing SNSCC. Analysis between the p16 positive and negative groups is comparable for age, gender, smoking status, tumour stage at the time of biopsy, and initial treatment. This is the first Canadian study to demonstrate a significant survival advantage with p16 overexpression, and therefore oncogenic HPV, in SNSCC.

Findings of this study indicate that p16 overexpressing SNSCC may behave similarly p16 overexpressed OPSCC [11-16]. This retrospective study supports further research into p16 overexpression, and HPV associated SNSCC. p16 overexpression is an adequate surrogate marker for HPV in the sinonasal cavity, supported by Bishop et al. (2013) who reported that p16 overexpression strongly correlated with the presence of HPV DNA in sinonasal tumors [27]. The current study outlines a need to delineate whether HPV-associated tumors possess distinct molecular features, as has been shown in HPV-associated OPSCC [11-16]. Further research in HPV-associated SNSCC may reveal variations in treatment response and prognosis.

Limitations of this current retrospective analysis include the small sample size, given both the limited population prevalence of SNSCC and that only tumors with p16 evaluation status were eligible for inclusion. In pathological evaluation, p16 staining was conducted at random by pathologists and without prior clinical indication. As such, there should be no inherent confounding by indication to the selection of those patients included in our analysis. Finally, the status of patient's exposure to other risk factors of SNSCC that may affect prognosis and survival was limited.

Future work will continue to validate these findings in larger patient populations, accounting for possible idiosyncrasies in this small cohort. Additionally, further molecular analysis distinguishing may reveal a pathobiological mechanism for survival differences. Finally, larger trials will characterize optimal treatment standards differentiated by p16 status, incorporating current findings as a crucial clinical parameter to guide treatment decisions.

5. Conclusions

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This population-based study is the first in the Canadian medical literature to demonstrate the statistically significant role of p16 overexpression in prognosis and survival of SNSCC. Further work in this area to delineate the effect of HPV on treatment, prognosis, and survival for SNSCC is essential for optimal patient care.

Author Contributions: Conceptualization, Jill Querney and David Côté; Data curation, Jill Querney and Jacob Wihlidal; Formal analysis, Jill Querney; Investigation, Jill Querney and Vincent Biron; Methodology, Jill Querney and David Côté; Project administration, Jacob Wihlidal; Resources, Vincent Biron; Supervision, David Côté; Validation, Adrian Mendez and Jacob Wihlidal; Visualization, Jacob Wihlidal and Fatemeh Ramazani; Writing – original draft, Jill Querney, Jacob Wihlidal and Fatemeh Ramazani; Writing – review & editing, Adrian Mendez, Jacob Wihlidal, Fatemeh Ramazani, Moses Fung and David Côté.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki and approved by the Health Research Ethics Board of the Alberta Cancer Committee (HREBA.CC-17-0431_REN1).

Informed Consent Statement: Informed consent was obtained from all subjects involved in the study.

Data Availability Statement: Not applicable.

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Conflicts of Interest: The authors declare no conflict of interest.

Appendix A

Table 2. Patient data and categorical analysis between p16 positive and p16 negative samples.

ID	p16	Age	Sex	Stage at Biopsy	Smoker	Initial Treatment
1	+	23	F	pT1N0	No	Surgery
2	+	70	F	pT1Nx	No	Surgery
3	+	61	F	pT3Nx	No	Surgery
4	+	48	M	pT4aNx	Yes	Surgery + Adjuvant C+RT
5	+	48	M	pT4bN0	Yes	Surgery
6	+	50	M	pT4aN1	Yes	Surgery + Adjuvant RT
7	+	61	M	pTisN0	No	Surgery
8	+	80	M	pT3N0	Yes	Surgery + Adjuvant RT
9	+	69	F	pT3Nx	Yes	Surgery
10	+	75	M	pT4aNx	Yes	Surgery + Adjuvant RT
11	+	71	F	pT4aNx	Yes	No treatment
12	-	55	M	pT4N0	Yes	Surgery + Adjuvant RT
13	-	67	M	pT1Nx	Yes	Surgery
14	-	26	M	pT4Nx	No	C+RT
15	-	89	F	pT4aN2b	No	Surgery + Adjuvant RT
16	-	59	M	T4bN1	Yes	C+RT + Adjuvant Surgery

C = chemotherapy; RT = radiation therapy

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