C-reactive Protein in HPV-Positive and HPV-Negative Oropharyngeal Cancer

Stephanie Johnson-Obaseki, MD, FRCSC, MPH1, Lisa Caulley, MD, FRCSC, MPH1, Martin Corsten, MD, FRCSC2, Geoffrey Liu, MD, FRCP3, Jim Dimitroulakos, PhD1, David Goldstein, MD, FRCSC3, Jonathan Irish, MD, FRCSC3, and Jennifer Rider, ScD, MPH4,5

Sponsorships or competing interests that may be relevant to content are disclosed at the end of this article.

Abstract
Objective. Evaluate serum C-reactive protein (CRP) in human papillomavirus (HPV)–positive oropharynx cancer as compared with HPV-negative oropharynx cancer and determine if CRP levels were associated with overall survival and/or recurrence-free survival.

Study Design. Prospective cohort study.


Subjects and Methods. Among patients with oropharynx cancer and confirmed HPV status, plasma CRP levels were measured with a high-sensitivity ELISA kit. Multivariable logistic regression analysis compared 4 categories of CRP (low, moderate, high, very high) between the HPV-positive and HPV-negative groups. Kaplan-Meier methods and Cox regression models were used to determine overall survival and recurrence-free survival by CRP level in both populations.

Results. Between 113 HPV-positive and 110 HPV-negative patients, CRP levels were significantly higher in the HPV-positive group, but these levels did not demonstrate a statistically significant dose-response trend. Higher CRP levels were also associated with reduced overall survival (P = .016) and recurrence-free survival (P < .001) within the HPV-negative group in univariable analysis; in multivariate analysis, the comparisons were not significantly different. Within HPV-positive oropharynx cancer, CRP levels were not significantly associated with overall survival or recurrence-free survival in univariable or multivariable analyses.

Conclusion. Circulating CRP was higher in HPV-positive versus HPV-negative oropharynx cancer. Among HPV-negative patients, higher CRP levels were associated with reduced survival.

Keywords
head and neck cancer, oropharynx, oropharyngeal, inflammation, inflammatory, C-reactive protein, human papillomavirus

Received October 3, 2017; revised June 28, 2018; accepted August 22, 2018.

Inflammation is known to have a causal relationship with many diseases, including those of the pulmonary, neurologic, cardiovascular, and autoimmune systems, as well as dementia and cancer.1 In addition, inflammation may have a reciprocal relationship in many of these diseases: inflammation contributes to the cause of the disease, but the disease itself then promotes an inflammatory environment, thereby worsening the disease state.1

In the 19th century, Rudolf Virchow first described the association between the development of cancer and inflammation; however, it was only in the last decade that a clear relationship was established between inflammation and tumorigenesis. An inflammatory microenvironment is essential for the initiation and progression of all tumors.2-6 In fact, inflammation was recently suggested as the seventh hallmark of cancer, after the 6 identified by Hanahan and Weinberg: self-sufficiency in growth signals, insensitivity to antigrowth signals, evasion of apoptosis, limitless replicative potential, sustained angiogenesis, and tissue invasion/metastasis.7

C-reactive protein (CRP) is an acute-phase protein produced by the liver. Serum CRP measurements are widely used as biomarkers of inflammation in the body. Elevated levels of serum CRP are associated with inflammatory conditions such as heart disease and certain autoimmune diseases. Elevated serum CRP levels were also found in many
cancers, such as prostate,\textsuperscript{9} breast,\textsuperscript{10} esophageal,\textsuperscript{11} lung,\textsuperscript{12,13} cervical,\textsuperscript{14} and pancreatic cancers.\textsuperscript{15} Several small studies demonstrated elevated serum CRP levels in some subsites of head and neck cancers (HNCs), such as the oral cavity and larynx.\textsuperscript{16,17}

Although CRP is a nonspecific marker of inflammation, it tends to be differentially affected by different disease states. CRP levels in bacterial infections are much higher than in viral infections. In the setting of cancer, CRP levels tend to be elevated in the range of 10,000 to 40,000 ng/mL.\textsuperscript{18}

HPV-positive HNCs are unique from other HNCs in that patients are diagnosed at a younger age, with females affected at a higher incidence than previously, and there is improved survival versus non-HPV HNCs.\textsuperscript{19-25} There is also a relative paucity of literature investigating the relationship between inflammation and HNCs as compared with other cancers. Many of these studies used markers of inflammation, such as circulating neutrophils and mono- and lymphocytes,\textsuperscript{26} neutrophil:lymphocyte ratio,\textsuperscript{27,28} platelet:lymphocyte ratio,\textsuperscript{29} and tumor-infiltrating lymphocytes.\textsuperscript{30,31} Very few studies specifically examined the relationship between CRP and HNC—most of which focused on oral cavity and larynx cancers. All these studies share a common thread in that increased markers of inflammation are associated with poorer prognosis. The present study is the first to our knowledge to investigate the difference in CRP levels between HPV-positive and HPV-negative oropharynx cancers (OPCs) and determine if CRP levels are associated with differential survival. As such, the purpose was to answer the following questions: Is there a difference in serum CRP levels in HPV-positive OPC versus HPV-negative OPC? Within HPV-positive and HPV-negative groups, is there a difference in survival associated with CRP levels?

**Methods**

In this prospective cohort study, patients with incident OPC of the tonsil and tongue base were recruited at the Princess Margaret Cancer Centre of the University Health Network (UHN; Toronto, Canada) between 2007 and 2010. Patients were recruited at their first visit to see the specialist team, which was after a diagnosis was made. The median time from biopsy to blood draw was 2 weeks (range, 1.2 to 3.8). As CRP has a constant half-life of 18 hours, a biopsy 2 weeks prior should have a minimal impact on the CRP levels at the time of sampling. Clinico-demographic data in the form of a patient-reported questionnaire and plasma specimens were collected. Of 405 patients with OPC recruited (79% recruitment rate), 386 had plasma EDTA samples collected prior to treatment initiation that had a needle-to-freezer time <2 hours, of which 356 had no evidence of distant visceral metastases (patients who were not being considered for radiation or surgery due to disease stage were excluded from analysis). Patients with autoimmune disease or infection or those on anti-inflammatories were excluded at the outset of the study (n = 2). Patients were classified as HPV positive if p16 (a well-characterized surrogate marker of HPV infection) was strongly and diffusely positive. Patients with unknown HPV status were excluded from the analysis. Of the remaining patients, 110 were known to be HPV negative, and all were chosen for analysis. As a comparator group, known HPV-positive OPC patients were distribution matched in a 1:1 ratio (n = 113) according to age, sex, and cumulative smoking. Plasma EDTA samples from both patient groups were sent to The Ottawa Hospital Research Institute (Ottawa, Canada), where CRP assays and statistical analyses were undertaken. Survival data from the hospital registry were supplemented with Ontario provincial cancer registry data. All patients had electronic patient record chart reviews, while tumors were staged with the American Joint Committee on Cancer TNM staging system.\textsuperscript{32} Institutional research ethics board approval was obtained at both UHN and The Ottawa Hospital.

All patients were assessed and treated by a multidisciplinary team. Stage I-III tumors were treated with unimodality treatment (radiation therapy [RT] alone) with an altered fractionation scheme (60 Gy in 25 fractions in 5 weeks or 70 Gy in 35 fractions in 6 weeks). Stage IV disease was treated with multimodality treatment (concomitant chemoradiotherapy) with cisplatin (100 mg/m\textsuperscript{2} on days 1, 22, and 43 and RT (70 Gy in 35 fractions over 7 weeks). Exceptions were made where patients were unable to receive chemotherapy (elderly, renal/hepatic/cardiac impairment, or poor performance status). For some patients, EGFR inhibitors were used instead of chemotherapy. Patients were treated similarly regardless of their HPV status. RT planning was completed via intensity-modulated RT. Follow-up was performed according to the guidelines of the National Comprehensive Cancer Network\textsuperscript{33}; patients were assessed for treatment response at 10 to 12 weeks following end of primary treatment and for decision making surrounding completion neck dissection. Following this, patients were assessed every 3 months for the first 2 years, every 4 months in the third year, and every 6 months in years 4 and 5, as well as annually thereafter.

**Serum High-Sensitivity CRP Quantification**

High-sensitivity CRP was quantified by employing the Quantikine ELISA Kit from R&D Systems (Minneapolis, Minnesota) following the manufacturer’s protocol in the obtained EDTA plasma samples. With this kit, the CRP detection range in plasma is 0.78 to 50 ng/mL (sensitivity, 0.022 ng/mL). Quantification of CRP levels was performed with the Microplate Reader Thermo Multiskan Ascent and Accent Software 2.4 (both from Life Technologies, Burlington, Canada) at 450 nm with a 570-nm correction wavelength.\textsuperscript{34}

**Statistical Analysis**

To get a sense of the distribution of CRP in the 2 groups, CRP was initially analyzed as a continuous variable. Next, to detect small differences in CRP between the groups, we first analyzed CRP in deciles of the HPV-negative group, with the HPV-positive group following the same cut points
as the HPV-negative group. From these deciles, we saw clear cut points between the groups where there was a difference of ≥10 patients, and using these natural cut points, we collapsed the deciles into 4 categories: low, intermediate, high, and very high. Next, we performed a univariable analysis of HPV-positive versus HPV-negative OPC using comparisons between these groups. Multivariable analysis was also undertaken taking into account age, sex, smoking status (continuous variable by pack years), body mass index (BMI), and T and N classification.

Time-to-event analyses were performed separately for HPV-negative and HPV-positive patients for the outcomes of overall survival (OS) and recurrence-free survival (RFS; duration of survival without relapse at a local, regional, or distant site). HPV-negative patients were grouped as described previously, whereas HPV-positive patients were divided into groups based on cut points available in the literature: normal (<1 mg/L), intermediate (1-10 mg/L), high (11-22 mg/L), and very high (>22 mg/L). We conducted univariable analyses with Kaplan-Meier curves and log-rank tests. Multivariable analyses were utilized with Cox regression models to estimate hazard ratios and 95% CIs, controlling for age, sex, smoking, BMI, and T and N stage (categorical). All statistical analyses were carried out with SAS 9.4 (SAS Institute, Cary, North Carolina).

Results

Patient Demographics

A total of 113 HPV-positive and 110 HPV-negative patient samples were obtained from the UHN laboratory for analysis. Patient samples were matched on age, sex, and smoking pack years, resulting in no significant differences in these variables between the groups (Table 1). The mean BMI was slightly higher in the HPV-positive group (26.9 vs 24.7 in the HPV-negative group, \( P = .001 \)). T stage was similar between the groups, but N stage was significantly higher in the HPV-positive group, which was expected as HPV-positive OPC has been well documented to occur at a later N stage than that of HPV-negative OPC (\( P = .010 \)).

Comparison of CRP between HPV-Positive and HPV-Negative Patients

We found no statistically significant difference in CRP levels between the HPV-positive and HPV-negative groups when CRP was analyzed as a continuous variable (Figure 1, Table 2). When patients were divided into 4 groups based on CRP levels (Table 3), we found that HPV status was statistically significantly associated with CRP levels (Table 4). For example, when the very high CRP group was compared with the low CRP group, the odds ratio was 4.22 (95% CI, 1.44-12.36). A multivariable logistic regression analysis was then performed to examine potential differences in CRP between the HPV-negative and HPV-positive groups, while controlling for age, sex, smoking, BMI, and T and N stage. The adjusted analysis revealed significantly higher CRP levels in the HPV-positive group (Table 4). For example, between the very high CRP group and the low CRP group, the odds ratio was 4.59 (95% CI, 1.34-15.79). However, as there was not a dose-response relationship, the test of trend across the categories was not statistically significant (\( P = .987 \)).
Survival Analysis

HPV-Negative OS. Univariable analysis with log-rank statistics and Kaplan-Meyer curves demonstrated a less favorable OS among the patients with higher CRP levels (Figure 2; \( P = .016 \)). In the multivariable Cox regression analysis controlling for age, sex, smoking, and T and N stage, the difference in OS by CRP levels demonstrated a significant difference between the low versus very high CRP groups (hazard ratio, 9.09; 95% CI, 1.06-78.15; \( P = .04 \)).

HPV-Negative RFS. Univariable analysis with log-rank statistics and Kaplan-Meyer curves demonstrated a less favorable RFS among the patients with higher CRP levels (Figure 3; \( P = .0002 \)). However, with the Cox regression analysis controlling for age, sex, smoking, and T and N stage, the difference in RFS by CRP levels did not maintain statistical significance (\( P = .089 \)).

HPV-Positive OS. Neither the univariable (Figure 4) nor multivariable regressions for OS by CRP level in the HPV-positive group were statistically significant.

HPV-Positive RFS. Similarly for RFS, neither the univariable (Figure 5) nor multivariable regressions by CRP level in the HPV-positive group were statistically significant.

Discussion

There is increasing evidence to suggest a role for chronic inflammation in tumorigenesis. This may arise from the pro-tumor actions of inflammatory cells to promote tumor cell proliferation and maintain a favorable environment for tumorigenesis. Alternatively, inflammation may lead to the subversion of the host response from desensitization of receptors to the overproduction of inflammatory chemokines.
and cytokines. The role of inflammatory biomarkers as clinical tools could have significant implications for prognostication and therapy in HNC management.

Our study represents the first analysis of the association between pretreatment CRP levels and OPCs. A categorical analysis of CRP levels by HPV status revealed a statistically significant difference in CRP levels between HPV-positive and HPV-negative OPCs, but these levels did not demonstrate a statistically significant dose-response trend. The elevated levels of CRP among HPV-positive patients may be due to the fact that virally mediated cancers promote a proinflammatory microenvironment or that persistent infection is more likely in the setting of increased inflammation. This concept was studied in the setting of cervical cancer, which shares HPV as an etiologic agent. Further investigation is needed to determine the significance of the higher CRP levels in HPV-positive OPC, as the survival impact remains unclear.

With the rising incidence of HPV-related HNC among younger, healthier patients, there is a growing imperative to establish a comprehensive understanding of the pathophysiology of HNCs. Hence, as a secondary analysis, this study sought to determine the impact of CRP on OS and RFS as stratified by HPV status in OPCs. For HPV-negative patients, univariable analysis of OS and RFS revealed significantly worse survival among patients with higher CRP levels. This was maintained after adjustment of other covariates for OS but became nonsignificant for RFS. This inverse correlation between CRP level and survival among HPV-negative patients is consistent with recent studies that identified pretreatment elevation of serum CRP as a prognostic indicator in oral, prostate, breast, esophageal, lung, cervical, and pancreatic cancers. Khandavilli et al demonstrated that a raised pretreatment CRP was associated with worsened OS in oral cavity squamous cell carcinoma. The predictive power of CRP levels was found to be increased when combined with tumor size and stage. Gallo et al demonstrated a correlation between CRP or interleukin 6 and survival in a heterogeneous HNC population that included only 3 patients with OPC. Multiple pre- and postoperative biomarkers were measured and provided evidence of a potential role for acute-phase proteins in the regulation of the complex host response to malignancies. Similarly, when Zeng et al investigated the effect of CRP levels on the prognosis of patients with locoregionally advanced laryngeal cancer, they found that CRP was an independent predictor of cancer-specific survival.
This study did not demonstrate any differences in OS or RFS in the HPV-positive group. As such, our results contrast the findings of Huang et al., despite the fact that the same patient population was used in both studies. In 2015, Huang et al. investigated the prognostic value of the pretreatment circulating neutrophil, monocyte, and lymphocyte count in this same OPC patient population. Their study results demonstrated that a high circulating neutrophil count and monocyte count independently predicted poorer OS and RFS, whereas a high circulating lymphocyte count predicted a higher RFS and a marginally better OS among HPV-positive patients with OPC. There was no evidence of this association for HPV-negative patients. The implications of high circulating neutrophil count in our HPV-positive versus HPV-negative patients remains unclear; as such, further studies are needed to investigate the role of host inflammation in HPV-related OPcs. To further understand the role of CRP in OPC, the association between CRP and other established biomarkers of inflammation in OPC should be investigated, including tumor-infiltrating lymphocytes, circulating neutrophil count, circulating monocyte count and circulating lymphocyte count.

It is important to note that our study was not powered to detect differences in survival between the groups; therefore, there may be differences in survival in the HPV-positive group that were not revealed due to our relatively small sample size. Also, it is extremely difficult to show differences in survival among HPV-positive patients, as the survival is so favorable and the event rate tends to be fairly low. A larger and more targeted study to determine differences in survival by CRP level is warranted to answer this question.

As HNCs create a significant global health and socioeconomic burden with >600,000 new cases annually and account for >$1 billion annually (2017 US dollars), the early detection and targeted management of HNCs by cost-effective methods are essential. As well, the possible prognostic significance of biomarkers in the selection of patients for immunotherapy must be considered. Blay et al. found high serum CRP levels to be adverse prognostic factors among patients with metastatic renal cell carcinoma; they also identified an adverse prognostic significance of high CRP levels among patients selected for interleukin 2 (IL-2) immunotherapy. Pretreatment CRP concentrations were higher for patients who experienced progressive disease after IL-2 treatment, and median survival from the beginning of IL-2 therapy for patients with high CRP levels was inferior as compared with those with low CRP levels. Further studies are necessary to determine the role of CRP as a marker of disease, especially early detection of recurrences and metastases as well as treatment response in OPcs.

One inherent limitation of this study is that CRP is a nonspecific inflammatory marker. This highlights the value of serial measurements of CRP as a biomarker in cancers, as multiple measurements may help to isolate and clarify the role of CRP in OPC. Future longitudinal studies of CRP may shed light on the role for CRP as a biomarker of OPC prognosis, progression, and treatment response. It is also important to recognize that the relationship between CRP and cancer is still somewhat unclear in terms of differentiating whether elevated levels of CRP mark the presence of cancer or the increased future risk of cancer. Understanding the cause-and-effect relationship between CRP and cancer would help to determine how to act on elevated CRP levels. For example, if there is a causal relationship whereby inflammation causes cancer, then intervening may alter the disease risk, but if it is a marker of the disease, then intervening will not change the disease risk. Future studies are needed to further delineate this causal relationship.

The recruitment rate (79%) for this study was sufficiently high to ensure confidence in the generalizability of the study findings. However, ideally, we would have completed an analysis of the patients who did not consent to the study to ensure that there was no significant selection bias. We were unable to perform such an analysis, as we were limited to what was approved by our ethics board. This is unfortunate, as understanding the clinical characteristics of patients who refused to participate could have provided additional insight into potential biases and should be considered in future studies.

In summary, the results of this cohort study demonstrate that circulating CRP is higher in HPV-positive OPC as compared with HPV-negative OPC. For HPV-negative patients, higher CRP levels were associated with reduced survival. As CRP is a readily available and economically feasible prognostic tool for many cancers, further study will be required to determine its role in prognostication in the setting of HPV-related OPC. In addition, further longitudinal clinical data on integration of biomarkers into oncologic disease management are needed.

Author Contributions
Stephanie Johnson-Obaseki, study conception/design, statistical and data analysis, writing and revising manuscript; Lisa Caulley, writing/revising manuscript, data/statistical analysis; Martin Corsten, study design, writing and revising manuscript; Geoffrey Liu, input into study design and acquisition of data, drafted the manuscript and revised it critically for important intellectual content; Jim Dimitroulakos, specimen analysis, writing and revising the manuscript; David Goldstein, data analysis and interpretation, drafted the manuscript and revised it critically for important intellectual content; Jonathan Irish, involved in study design and data acquisition, drafted the manuscript and revised it critically for important intellectual content; Jennifer Rider, study design, statistical analysis, writing/revising the manuscript.

Disclosures
Competing interests: None.
Sponsorships: None.
Funding source: Aurora Research Institute educational grant.

References


