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Association Between HPV Circulating Tumor DNA and Prognostic Inflammatory Indices in Oropharyngeal Squamous Cell Carcinoma: A Pilot Study

Ryan S. Ziltzer, MD, MPH[1], Zulkifl I. Jafary, BA[2], Connor Hunt, BS[2], Iraj Hasan, BA[2], and Meghan T. Turner, MD, FACS[1]

Abstract

Circulating tumor DNA (ctDNA) has been developed as a marker of tumor burden in human papillomavirus (HPV)-positive oropharyngeal squamous cell carcinoma (OPSCC). Inflammatory indices are also increasingly being used as prognostic surrogate markers in solid tumors, including head and neck cancers. The relationship between ctDNA levels and inflammatory indices has not been studied in HPV-associated OPSCC. We hypothesize that higher levels of inflammation are associated with higher ctDNA levels. Herein, we demonstrate an association between high pretreatment ctDNA levels and specific inflammatory indices, which may be lower-cost surrogate markers of high HPV ctDNA levels and may act as a surrogate marker for the body's immune response to HPV-positive OPSCC.

Keywords

circulating tumor DNA, head and neck cancer, HPV, inflammatory indices, oropharyngeal squamous cell carcinoma

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Oropharyngeal squamous cell carcinoma (OPSCC) caused by human papillomavirus (HPV) has seen an epidemic rise in the last decade and has surpassed cervical cancer as the most common HPV-related cancer.[1] Early HPV-associated OPSCC has survival rates in excess of 90%, no matter the treatment.[2,3] However, a small percentage of patients experience delayed treatment failures.

Recently, circulating tumor DNA (ctDNA) assays that identify E6 and E7 protein amplicons produced by high-risk HPV strains in cell-free plasma have been developed as biomarkers for active HPV cancers.[4] Higher ctDNA levels have been associated with worse disease burden.[5-7] Persistently elevated levels posttreatment are associated with worse prognosis.[4,8]

Inflammatory indices are also being investigated as prognostic surrogate markers in various solid tumors,[9,10] including head and neck cancers.[11-13] Inflammatory indices are calculated from complete blood cell count (CBC) with differential laboratory values and reflect systemic inflammation levels. In OPSCC, several studies have demonstrated worse overall survival (OS) and disease-free survival/recurrence-free survival (DFS/RFS) with higher systemic inflammatory indices.[14-17]

Given that ctDNA acts as a biomarker for active HPV-related OPSCC that is detected in circulating plasma, it would be reasonable to hypothesize that there might be an association with systemic immune response and, therefore, systemic inflammatory indices. To date, no study has looked at the relationship between ctDNA levels and systemic inflammatory indices. This pilot study examines whether pretreatment inflammatory indices are associated with pretreatment ctDNA levels in HPV-positive OPSCC.

[1]Department of Otolaryngology–Head and Neck Surgery, West Virginia University, Morgantown, West Virginia, USA
[2]West Virginia University School of Medicine, Morgantown, West Virginia, USA

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Corresponding Author:
Meghan T. Turner, MD, FACS, Department of Otolaryngology–Head and Neck Surgery, West Virginia University, 1 Medical Center Drive Rm PO Box 9200, Morgantown, WV 26506, USA.
Email: meghan.turner@hsc.wvu.edu

Materials and Methods

This was a retrospective pilot study of HPV OPSCC patients with pretreatment levels of ctDNA (NavDx, Naveris Laboratories) identified from 2021 to 2023 at an academic hospital. Institutional review board approval was obtained from the WVU Office of Human Research Protections before the start of data collection (Protocol#2201496852). All patient data were collected via retrospective chart review and kept in a deidentified data sheet.

The population was divided into two cohorts and classified as either "high" or "low" from the calculated median ctDNA level. Absolute cell counts from CBC with differential near the time of ctDNA blood draw were used to calculate four prognostic inflammatory indices: neutrophil-to-lymphocyte ratio (NLR), platelet-to-lymphocyte ratio (PLR), systemic immune inflammation index (SII = platelet × neutrophil/lymphocyte), and systemic inflammation response index (SIRI) = neutrophil × monocyte/lymphocyte). Similarity in characteristics—age, tumor stage (T-stage), and nodal stage (N-stage)—between ctDNA level groups was assessed using Fisher's exact chi-square. Associations between high or low ctDNA levels and inflammatory indices were assessed using two-sample *t* tests.

Results

This pilot analysis included 16 patients who had pretreatment ctDNA and CBC with differential lab values. Most patients were male (15, 93.75%). The average age was 65.11 years (SD = 9.53). Most patients had T1 and T2 tumors (14, 87.5%). Most patients had N1 neck disease (11, 68.75%). Study population and demographic data are available in **Table 1**.

The median pretreatment ctDNA level was 643.5 copies/μL. Age, T-stage, and N-stage were similar between high and low ctDNA patients ($P > .05$). High ctDNA levels, compared to low ctDNA levels, were associated with higher average NLR (3.09 vs 1.75, $P = .003$) and higher average SIRI (1.54 vs 1.07, $P = .04$). The results of comparisons between groups with high and low ctDNA are available in **Table 2**.

Discussion

This pilot study examines a novel proof-of-concept use of inflammatory indices in HPV-related OPSCC and suggests that patients with high ctDNA levels may have higher systemic inflammation. This finding has benefits that include the use of routine, inexpensive laboratory testing to provide prognostic information. This may reflect the immune response to ctDNA and may be a potential surrogate marker for worse disease burden.

In general, worse disease burden is associated with worse prognosis in HPV-related disease, as reflected in AJCC staging.[18] ctDNA has recently been studied as a marker for HPV-related tumor burden in OPSCC.[19] A study by Lam et al noted higher median preoperative ctDNA levels with higher pathologic tumor stage in patients undergoing surgery.[5] A study by Rettig et al recently demonstrated that higher ctDNA levels were associated with "higher clinical nodal stage" and "diameter of the largest lymph node" on preoperative imaging.[6] Finally, another study by Hanna et al showed that median ctDNA scores were "higher with an increasing per-patient number of metastatic sites."[7]

Table 2. Inflammatory Indices in High and Low ctDNA Patients

Index	Low ctDNA Mean (SD)	High ctDNA Mean (SD)	*P* value
NLR	1.75 (0.50)	3.09 (0.94)	.003*
PLR	111.19 (30.93)	124.35 (60.36)	.58
SII	427.34 (176.20)	587.77 (260.48)	.17
SIRI	1.07 (0.46)	1.54 (0.30)	.04*

Abbreviations: ctDNA, circulating tumor DNA; NLR, neutrophil-to-lymphocyte ratio; PLR, platelet-to-lymphocyte ratio; SD, standard deviation; SII, systemic immune inflammation index; SIRI, systemic inflammation response index.
*$P < .05$.

Table 1. Comparing Clinical Characteristics in Low and High ctDNA Patients

Variables	Low ctDNA (n = 7)	High ctDNA (n = 9)	*P* value
Characteristics			
ctDNA, mean (SD)	237.14 (203.82)	11,554.78 (14,772.51)	>.001*
Age, mean (SD)	67.6 (9.6)	63.2 (9.6)	.47
T-stage, n (%)			>.9
T1	3 (43%)	4 (44%)	
T2	3 (43%)	4 (44%)	
T3	1 (14%)	1 (11%)	
N-stage, n (%)			.31
N1	6 (86%)	5 (56%)	
N2	1 (14%)	4 (44%)	

Abbreviations: ctDNA, circulating tumor DNA; N-stage, nodal stage; SD, standard deviation; T-stage, tumor stage.
*$P < .05$.

Recent evidence suggests that inflammatory indices are associated with worse prognosis in OPSCC. A systematic review demonstrated that a high NLR has been associated with worse OS, RFS, and DFS.[14,17] Other studies have found that higher PLR and the SII are associated with worse survival.[15,16]

Within the tumor microenvironment, a predominance of neutrophils and monocytes is associated with immunosuppressive states by producing tumor cell death, tumor antigen release (eg, ctDNA), and presentation of antigens to regulatory and CD4+ T cells involved in cancer immune escape.[20,21] This might explain why higher ctDNA levels are associated with higher NLR and SIRI levels. Platelets are thought to shield tumor cells from immune-mediated cell death.[22] This may be why there was no association with ctDNA levels and PLR and SII.

To our knowledge, this is the first study demonstrating a relationship between pretreatment ctDNA levels and the pretreatment inflammatory indices, which might represent a lower-cost surrogate indicator of high HPV ctDNA levels. Used in combination, the two might provide improved prognostic information for patients with HPV-positive OSPCC. The limitations include small sample size, retrospective study design, and a lack of available validated classifications of high and low ctDNA levels. Future larger-scale studies of ctDNA and inflammatory indices, posttreatment associations, and correlates with survival/prognosis are needed.

Author Contributions

Ryan S. Ziltzer, study design, data interpretation/statistical analysis, manuscript writing; **Zulkifl I. Jafary**, data collection, critical revision of manuscript; **Connor Hunt**, data collection, critical revision of manuscript; **Iraj Hasan**, data collection, critical revision of manuscript; **Meghan T. Turner**, study concept and design, data interpretation, manuscript writing and critical revision.

Disclosures

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ORCID iD

Ryan S. Ziltzer http://orcid.org/0000-0002-1952-0626
Meghan T. Turner http://orcid.org/0000-0001-8621-6168

References

1. Van Dyne EA, Henley SJ, Saraiya M, Thomas CC, Markowitz LE, Benard VB. Trends in human papillomavirus–associated cancers—United States, 1999–2015. *MMWR Morb Mortal Wkly Rep*. 2018;67(33):918-924. doi:10.15585/mmwr.mm6733a2
2. Yom SS, Torres-Saavedra P, Caudell JJ, et al. Reduced-dose radiation therapy for HPV-associated oropharyngeal carcinoma (NRG Oncology HN002). *J Clin Oncol*. 2021;39(9):956-965. doi:10.1200/JCO.20.03128
3. Ferris RL, Flamand Y, Weinstein GS, et al. Phase II randomized trial of transoral surgery and low-dose intensity modulated radiation therapy in resectable p16+ locally advanced oropharynx cancer: an ECOG-ACRIN Cancer Research Group Trial (E3311). *J Clin Oncol*. 2022;40(2):138-149. doi:10.1200/JCO.21.01752
4. Chera BS, Kumar S, Shen C, et al. Plasma circulating tumor HPV DNA for the surveillance of cancer recurrence in HPV-associated oropharyngeal cancer. *J Clin Oncol*. 2020;38(10):1050-1058. doi:10.1200/JCO.19.02444
5. Lam D, Sangal NR, Aggarwal A, et al. Preoperative circulating tumor HPV DNA and oropharyngeal squamous cell disease. *JAMA Otolaryngol Head Neck Surg*. 2024;150(5):444. doi:10.1001/jamaoto.2024.0350
6. Rettig EM, Wang AA, Tran NA, et al. Association of pretreatment circulating tumor tissue–modified viral HPV DNA with clinicopathologic factors in HPV-positive oropharyngeal cancer. *JAMA Otolaryngol Head Neck Surg*. 2022;148(12):1120. doi:10.1001/jamaoto.2022.3282
7. Hanna GJ, Jabalee J, Lukens JN, et al. Circulating tumor tissue modified viral (TTMV)-HPV DNA in recurrent, metastatic HPV-driven oropharyngeal cancer. *Oral Oncol*. 2024;158:107002. doi:10.1016/j.oraloncology.2024.107002
8. Routman DM, Kumar S, Chera BS, et al. Detectable postoperative circulating tumor human papillomavirus DNA and association with recurrence in patients with HPV-associated oropharyngeal squamous cell carcinoma. *Int J Radiat Oncol Biol Phys*. 2022;113(3):530-538. doi:10.1016/j.ijrobp.2022.02.012
9. Chao B, Ju X, Zhang L, Xu X, Zhao Y. A novel prognostic marker systemic inflammation response index (SIRI) for operable cervical cancer patients. *Front Oncol*. 2020;10:766. doi:10.3389/fonc.2020.00766
10. Chen Z, Wang K, Lu H, et al. Systemic inflammation response index predicts prognosis in patients with clear cell renal cell carcinoma: a propensity score-matched analysis. *Cancer Manag Res*. 2019;11:909-919. doi:10.2147/CMAR.S186976
11. Li Q, Yu L, Yang P, Hu Q. Prognostic value of inflammatory markers in nasopharyngeal carcinoma patients in the intensity-modulated radiotherapy era. *Cancer Manag Res*. 2021;13:6799-6810. doi:10.2147/CMAR.S311094
12. Song F, Cai H, Liao Y, et al. The systemic inflammation response index predicts the survival of patients with clinical T1-2N0 oral squamous cell carcinoma. *Oral Dis*. 2022;28(3):600-610. doi:10.1111/odi.13782
13. Lin J, Chen L, Chen Q, et al. Prognostic value of preoperative systemic inflammation response index in patients with oral squamous cell carcinoma: propensity score-based analysis. *Head Neck*. 2020;42(11):3263-3274. doi:10.1002/hed.26375
14. Justesen MM, Jakobsen KK, Bendtsen SK, et al. Pretreatment neutrophil-to-lymphocyte ratio as a prognostic marker for the outcome of HPV-positive and HPV-negative oropharyngeal squamous cell carcinoma. *Viruses*. 2023;15(1):198. doi:10.3390/v15010198
15. Song Y, Cheng D, Luo Y, et al. Dynamic changes of hematological indices in oropharyngeal cancer patients

treated with radiotherapy. *Acta Otolaryngol*. 2022;142 (9-12):705-711. doi:10.1080/00016489.2022.2140823

16. Brewczyński A, Jabłońska B, Mazurek AM, et al. Comparison of selected immune and hematological parameters and their impact on survival in patients with HPV-related and HPV-unrelated oropharyngeal cancer. *Cancers*. 2021;13(13):3256. doi:10.3390/cancers13133256
17. Rodrigo JP, Sánchez-Canteli M, Triantafyllou A, et al. Neutrophil to lymphocyte ratio in oropharyngeal squamous cell carcinoma: a systematic review and meta-analysis. *Cancers*. 2023;15(3):802. doi:10.3390/cancers15030802
18. American Joint Committee on Cancer. *AJCC Cancer Staging Manual*. 8th ed., corrected at 3rd printing. Amin MB, Greene FL, Edge SB, eds. AJCC, American Joint Committee on Cancer; 2017.
19. Veyer D, Wack M, Mandavit M, et al. HPV circulating tumoral DNA quantification by droplet-based digital PCR: a promising predictive and prognostic biomarker for HPV-associated oropharyngeal cancers. *Int J Cancer*. 2020; 147(4):1222-1227. doi:10.1002/ijc.32804
20. Elmusrati A, Wang J, Wang CY. Tumor microenvironment and immune evasion in head and neck squamous cell carcinoma. *Int J Oral Sci*. 2021;13(1):24. doi:10.1038/s41368-021-00131-7
21. Kallinger I, Rubenich DS, Głuszko A, et al. Tumor gene signatures that correlate with release of extracellular vesicles shape the immune landscape in head and neck squamous cell carcinoma. *Clin Exp Immunol*. 2023;213(1):102-113. doi:10.1093/cei/uxad019
22. Peltanova B, Raudenska M, Masarik M. Effect of tumor microenvironment on pathogenesis of the head and neck squamous cell carcinoma: a systematic review. *Mol Cancer*. 2019;18(1):63. doi:10.1186/s12943-019-0983-5