Circulating Tumor DNA in Head and Neck Cancer

Subjects: Otorhinolaryngology
Contributor: Andrew Birkeland

Head and neck cancer remains a challenging and deadly disease as it is often identified in more advanced stages due to limitations in screening and surveillance. Circulating tumor DNA (ctDNA) has the potential to improve outcomes by enhancing screening, early diagnosis, and surveillance in head and neck cancer patients.

Keywords: ctDNA ; cancer

1. ctDNA Utility in HPV-Positive Head and Neck Cancer

1.1. HPV Characterization

The Centers of Disease Control and Prevention estimates that human papillomavirus (HPV) now accounts for 70% of oropharyngeal squamous cell cancer (OPSCC) in the United States [1] .HPV is spread mainly through sexual contact and exerts its carcinogenic effect by its oncogenes E6 and E7, which inactivate host tumor suppressor proteins p53 and pRb, respectively [2]. The importance of HPV status in OPSCC is established by studies demonstrating higher response rates to treatment and longer survival in patients with HPV-positive OPSCC, compared to patients with HPV-negative tumors [3][4]. Currently, the diagnosis of HPV-positive OPSCC is made by examining cytology specimens for either the presence of HPV DNA through polymerase chain reaction (PCR) or in situ hybridization, or through detecting HPV surrogate markers such as host p16 overexpression, demonstrated through immunohistochemistry [5].

1.2. HPV ctDNA as a Biomarker for Screening and Diagnosis

Circulating tumor DNA (ctDNA) detection methods may offer an alternative method for diagnosing HPV-positive head and neck squamous cell carcinoma (HNSCC) (**Table 1**). In a prospective observational study, Siravegna et al. demonstrated that detection of HPV ctDNA may offer a noninvasive and cost-effective diagnostic approach for HPV-positive HNSCC with improved accuracy and reduced time to diagnosis [6]. A total of 61 patients with new or suspected diagnosis of untreated HNSCC were enrolled, as well as 70 HPV-negative controls. All patients with HNSCC underwent a standard clinical workup, which included fine needle aspiration and/or tissue biopsy of the primary tumor. The diagnostic success rate of the first diagnostic attempt was 72% with 28% of patients requiring a second diagnostic attempt with tumor biopsy to determine diagnosis. Conversely, serum HPV ctDNA detection for diagnosing HPV-positive HNSCC had a sensitivity of 98.4%, specificity of 98.6%, positive predictive value (PPV) of 98.4%, and negative predictive value (NPV) of 98.6%. When the composite performance of the standard clinical workup on first diagnostic attempt was compared to HPV ctDNA on the first diagnostic attempt, HPV ctDNA demonstrated improved diagnostic accuracy. Next, the authors conducted cost modeling comparing standard of care pathways with scenarios where HPV ctDNA was the diagnostic of choice. They estimated savings of 36–38% (USD 6227–USD 6667) per patient with HPV ctDNA diagnostics. Additionally, with existing molecular diagnostic turnaround times of 5 days, the authors estimated an HPV ctDNA diagnostic approach to shorten time to diagnosis by 63% (26 days). HPV ctDNA was additionally found to possess high sensitivity, even in a cohort with low disease burden (75% of patients with Stage I OPSCC), furthering interest as a screening tool.

Table 1. Key studies examining HPV ctDNA as a biomarker for HPV-positive HNSCC.

Reference	Study Design	Sample Size	Findings/Strengths	Limitations
[7]	**Prospective**	**115**	- HPV ctDNA plasma detection has high NPV and PPV for disease recurrence surveillance - Phase II clinical trial - Multi-institutional study	- 29 patients did not have pre-treatment blood samples available
[6]	**Prospective**	**140**	- Demonstrates diagnostic capacity of HPV ctDNA testing as cost-effective with shorter diagnostic interval - Prospectively conducted	- Small sample size - Observational in design - Single institutional study
[8]	**Retrospective**	**112**	- First report to demonstrate HPV ctDNA detection years prior to cancer diagnosis - HPV ctDNA detection demonstrated high specificity for diagnosis of HPV-positive cancer	- Small sample size of only 12 cases - Retrospective design - Single-institutional study
[9]	**Cross-sectional analysis**	**408**	- HPV ctDNA testing in plasma had 100% specificity in healthy people - Large sample size	- Limited to single timepoint - Single institutional study
[10]	**Prospective**	**35**	- HPV ctDNA testing increases accuracy of post-treatment surveillance when combined with PET-CT imaging	- Small sample size - Single institutional study
[11]	**Prospective**	**103**	- Identified a favorable and unfavorable clearance profile that can predict CRT treatment response - Demonstrated utility of HPV ctDNA load to select patients for de-intensified therapy - Multi-institutional study	- Limited follow up
[12]	**Prospective**	**16**	- Serial HPV ctDNA loads can be used to measure treatment response with potential for guiding treatment intensification/deintensification	- Small sample size - HPV ctDNA only detected in 75% of patients with HPV-positive OPSCC
[13]	**Prospective**	**33**	- Clearance kinetics of HPV ctDNA can be used to identify patients at increased risk of recurrence and those who may benefit from adjuvant treatment.	- Small sample size - Single institutional study - Short follow-up

Reference	Study Design	Sample Size	Findings/Strengths	Limitations
[14]	Prospective	159	- Post-op HPV ctDNA levels have prognostic value for RFS and OS	- Pre-op to post-op HPV ctDNA level comparisons to a small subset of patients - Post-op blood collections for HPV ctDNA analysis collected at varying timepoints affecting understanding of ctDNA kinetics and quantity

RFS = Recurrence-free survival; OS = Overall Survival.

Several barriers for screening for HPV-positive OPSCC have been identified, including its relatively low overall incidence, rendering even ideal biomarkers with low PPV [15]. Additionally, in the way cervical cancer possesses an identifiable precursor lesion for screening, nothing similarly has been described for OPSCC. However, in a retrospective case–control study, Rettig et al. have shown that HPV ctDNA detection can occur several years prior to the diagnosis of HPV-positive OPSCC, suggesting HPV ctDNA positivity could serve as a surrogate precursor lesion [8]. Of the 10 patients with HPV-positive OPSCC enrolled, 3 had early detectable HPV ctDNA in plasma collected at a median time of 30.5 months prior to diagnosis. Neither the cases with HPV-negative OPSCC nor any of the 100 healthy controls had detectable HPV ctDNA in their plasma. While the generalizability of these findings is limited by the low number of cases, these findings demonstrate for the first time that HPV ctDNA can be detected in plasma years before a clinical diagnosis of HPV-positive OPSCC. The authors also demonstrated that HPV ctDNA can have high specificity with zero false positives reported. A cross-sectional analysis also found similar specificity for plasma-derived HPV ctDNA [9]. The authors enrolled 408 healthy participants without HNC but at heightened risk for HPV-related cancer, as determined by lifestyle factors. PCR conducted on plasma samples from participants did not detect any oncogenic HPV ctDNA.

1.3. HPV ctDNA as a Biomarker for Surveillance

Studies have begun examining the ability of HPV ctDNA plasma presence to detect disease recurrence in HPV-positive OPSCC with promising accuracy [16][17]. In a prospective clinical trial of 115 HPV-positive OPSCC patients, Chera et al. demonstrated that two consecutive positive HPV ctDNA blood tests during posttreatment surveillance was highly indicative of disease recurrence [7]. After a median follow-up time of 23 months, 15 patients developed biopsy-proven recurrence, all of whom had two consecutively positive HPV ctDNA tests during surveillance, with a sensitivity and specificity 100% and 99%, respectively. Another promising result was that the median lead time from the first positive HPV ctDNA to biopsy-proven recurrence was 3.9 months.

PET-CT imaging has remained a controversial surveillance modality, as it has yet to show survival advantage, and in some studies has been shown to have low PPV for detecting locoregional failure in HPV-positive OPSCC [18][19]. Tanaka et al. demonstrated that concomitant HPV ctDNA blood tests with PET-CT imaging, however, could improve recurrent/residual disease detection [10]. A total of 35 patients with HPV-positive OPSCC were enrolled in this prospective cohort study after completing chemoradiotherapy. After a median follow-up of 21 months, 9 patients had treatment failures. PET-CT imaging that displayed incomplete metabolic response had a 4.7-fold increase in risk of residual disease compared to patients who had complete metabolic response. However, with combined imaging and liquid biopsy results, positive HPV ctDNA levels and incomplete metabolic response on PET-CT portended a 138.8-fold increased risk of residual disease when compared to patients with non-detectable HPV ctDNA levels and incomplete metabolic response on PET-CT. Another study with a small cohort found similar improvement in the detection ability of post-chemoradiotherapy residual disease with combined PET-CT imaging and HPV ctDNA detection [20].

Other studies have begun determining the absolute quantification of HPV ctDNA levels in plasma specimens and analyzing its kinetic clearance pattern to predict recurrent/residual disease. Chera et al. recruited 103 patients with HPV-positive OPSCC who had undergone chemoradiotherapy in a multi-institutional prospective biomarker trial [11]. The authors found that patients with a baseline HPV ctDNA plasma level of >200 copies/mL and who had greater than 95% of HPV ctDNA clearance by week 4 post-treatment had a greater likelihood of disease control. Elsewhere, Haring et al. suggest that the percent change in HPV ctDNA levels during chemotherapy correlates with the radiographically

determined treatment response [12]. The authors demonstrated that HPV ctDNA levels showing an increase greater than 60% between baseline and cycle 3 of chemotherapy were predictive of progressive disease with a sensitivity and specificity of 89%. Post-operative HPV ctDNA levels have also been shown to predict residual disease risk. O'Boyle et al. showed that post-operative day 1 HPV ctDNA plasma levels of 1 copy/mL correlated with the lowest risk of residual disease, while 100 copies/mL correlated with higher incidence of pathologic risk factors such as extranodal extension and number of lymph nodes involved; these findings are also supported in another study by Routman et al. [13][14]. While future studies are needed to validate these findings, they do provide encouraging glimpses into how HPV ctDNA can potentially serve as a biomarker for guiding personalized treatment decisions, such as the need for adjuvant therapy or treatment deintensification.

2. ctDNA Utility in EBV-Associated Nasopharyngeal Carcinoma

2.1. EBV Characterization

The Epstein–Barr virus has been associated with several different malignancies, including nasopharyngeal carcinoma (NPC) [21][22]. It has been determined to affect around 85–95% of the healthy population and has been endemically linked with NPC in Southeast Asia [16]. Unfortunately, NPC is frequently diagnosed at later stages due to the inaccessible nature of the post-nasal space and often atypical presentation, leading to poorer patient outcomes [17].

2.2. EBV ctDNA as a Biomarker for Screening

The role for plasma EBV ctDNA load in the detection and screening utility of NPC has been well-characterized in the endemic literature [23][24]. It continues to be a role vigorously investigated (**Table 2**). The landmark prospective investigation conducted by Lo et al. found elevated EBV ctDNA loads in 55/57 (96.0%) patients with NPC compared to 3/43 (7.0%) of controls, establishing the value of plasma EBV ctDNA as a biomarker for screening NPC [25]. Similar results were reproduced in a non-endemic population but with a lower reported sensitivity (75.0%) [26]. Since then, several other EBV-associated biomarkers have been studied, including EBV viral capsid antigen and EBV early antigen IgA serology. Although the effectiveness of these other biomarkers has been inconsistently reported in the literature, they may prove to be beneficial in the detection of earlier stages of NPC [27][28][29]. These alternative biomarkers are important to consider, since EBV ctDNA load may not be as sensitive in detecting earlier compared to later-stage NPC [30]. On the other hand, several large prospective investigations in endemic areas have reported that the overall sensitivity and specificity of EBV ctDNA load in screening for NPC to be quite promising: at 86.8–97.1% and 90.0–98.6%, respectively [30][31]. Miller et al. reported through a hypothetical cohort that the combined usage of EBV ctDNA load and EBV serology would be a cost-effective option that could improve the 10-year overall survival from 71.0% to 86.3%, suggesting a potential advantage in combining these screening modalities [32]. However, more consistent methodological means are still needed to reduce inter-laboratory procedural variabilities, including DNA extraction protocols, and set EBV ctDNA load screening cutoff values [33][34].

Table 2. Key studies examining EBV ctDNA as a biomarker for EBV-associated NPC.

Reference	Study Design	Sample Size	Findings/Strengths	Limitations
[22]	**Prospective**	**1363**	- EBV ctDNA detectable group had a 10-fold higher incidence for NPC than undetectable group - Large sample size	- Did not retest or monitor EBV DNA fluctuation - Endemic population
[29]	**Prospective**	**523**	- EBV cfDNA load levels had poorer performance in screening for NPC than EBV IgA titers	- Endemic population - Only first-degree family members of NPC patients

Reference	Study Design	Sample Size	Findings/Strengths	Limitations
[30]	**Prospective**	**773**	- Detectable EBV ctDNA levels had lower sensitivity for screening for early stage NPC than advanced stage - Large study population	- Endemic population - Not all high-risk patients underwent diagnostics
[31]	**Prospective**	**20,174**	- EBV ctDNA detection in plasma samples had a sensitivity and specificity of 97.1% and 98.6% in screening for NPC - Large sample size - 2 different measurements to confirm EBV ctDNA	- Endemic population - Male only - Short 2-year follow-up interval
[35]	**Retrospective**	**480**	- Undetectable EBV ctDNA levels before treatment was associated with earlier T and N classification NPC	- Retrospective design - Single-institutional study
[36]	**Retrospective**	**278**	- After induction chemotherapy, detectable EBV ctDNA levels were associated with worse 3-year OS, DMFS, and DFS than undetectable levels	- Endemic population - Single-institutional study
[37]	**Retrospective**	**637**	- Pre-treatment EBV ctDNA loads >1500 copies/mL and post-treatment detectable EBV ctDNA were both associated with higher risk for recurrence and mortality	- Endemic population - Retrospective - Limited follow-up
[38]	**Retrospective**	**4469**	- Patients with large EBV ctDNA load had higher tendency for distant metastases - Large sample size	- Only pre-treatment EBV DNA measured
[39]	**Retrospective**	**1124**	- EBV ctDNA load > 4000 copies/mL during chemotherapy and IMRT treatment was an independent risk factor for OS - Large sample size	- Single-institutional study - Patients with heart, liver, renal, and/or hematologic comorbidities were excluded
[40]	**Prospective**	**260**	- Undetectable EBC ctDNA at 8 weeks and 6 months post-IMRT was associated with longer 3-year survival endpoints	- Endemic Population

OS = Overall survival; DMFS = Distant metastasis-free survival; DFS = Disease-free survival; IMRT = Intensity-modulate radiation therapy.

2.3. EBV ctDNA as a Biomarker for Surveillance and Prognosis

In addition to being positively correlated to clinical staging, EBV ctDNA load has also been extensively studied as an independent factor in monitoring treatment response [35][41][42][43]. To et al. determined that the median half-life of EBV DNA during surgical resection was 139 min, proving that EBV ctDNA is derived from the NPC tumor body [44]. Furthermore, the clearance of EBV ctDNA after chemoradiotherapy has been well documented in the literature and has been used as an indicator for treatment response [36][37][42]. Resurgences in EBV ctDNA levels after curative therapy have been associated with disease recurrence or residual malignancy, which suggests a possible use for EBV ctDNA measurements in disease surveillance [23][41][45]. Even when measured before treatment initiation, EBV ctDNA levels were significantly associated with metastatic disease [38][46]. In a meta-analysis of 16 pooled studies, Liu et al. found that the pooled hazard ratio for both locoregional recurrence and distant metastases in patients with higher pre-treatment EBV ctDNA levels were 3.12 and 3.68, respectively [41]. It should be mentioned that there were several different cutoffs for higher pre-treatment EBV ctDNA for the studies in this meta-analysis, ranging from 307 to 20,000 copies/mL. As with screening, further standardization of EBV ctDNA cutoff values and inter-laboratory methodological means has yet to be established.

EBV ctDNA levels are negatively correlated with survival outcome measures. Detectable pre-treatment plasma EBV ctDNA was found to be associated with poorer survival outcomes when compared to undetectable cases [35][39][42][47]. This trend has also been demonstrated in non-endemic investigations [34][48]. Additionally, several investigations found that even among cases with detectable EBV ctDNA, higher levels of plasma EBV ctDNA were associated with poorer survival outcomes. It should be noted that there was a high amount of variability regarding the cutoffs for higher levels of EBV DNA, with a range between 1500 and 7000 copies/mL [36][37][39][42][43]. In Liu et al.'s meta-analysis, higher EBV ctDNA loads were associated with poorer overall survival (OS) and disease-free survival (DFS) with hazard ratios of 3.0 and 2.4, respectively [41]. Several studies have also found mid-treatment and post-treatment detectable EBV ctDNA levels to be associated with poorer survival outcomes [36][37][42][49]. While there was a large overlap between patients with detectable mid and post-treatment EBV ctDNA levels, patients with increased mid-treatment but undetectable post-treatment EBV ctDNA levels were still found to have worse outcomes than individuals with undetectable mid-treatment levels [49]. Additionally, Chan et al. found patients undergoing radiotherapy with EBV ctDNA half-lives of ≥15 days to have significantly poorer OS, progression-free survival (PFS), and distant metastasis-free survival (DMFS) than those with more expedient clearance, suggesting a temporal relationship between viral DNA clearance and survival outcomes [50]. Although radiotherapy was found to decrease EBV ctDNA levels and improve survival outcomes in patients with NPC, the data concerning chemotherapy are mixed [40][51]. These data show that EBV ctDNA may play a significant role in determining patient prognosis, for both survival outcomes and disease relapse or metastases.

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