Received: 6 December 2023 | Revised: 20 June 2024 | Accepted: 30 June 2024

DOI: 10.1002/hed.27866

ORIGINAL ARTICLE

WILEY

# Circulating tumor HPV DNA in the management of HPV+ oropharyngeal cancer and its correlation with MRI

**Flaminia Campo PhD**[1] | **Francesca Paolini PhD**[2,3] | **Valentina Manciocco PhD**[1] | **Silvia Moretto MD**[1] | **Barbara Pichi MD**[1] | **Claudio Moretti MD**[1] | **Giovanni Blandino MD**[4] | **Valentina De Pascale MD**[5] | **Maria Benevolo MD**[6] | **Fulvia Pimpinelli MD**[7] | **Antonello Vidiri MD**[8] | **Simona Marzi MD**[8] | **Sergio Ruggiero MD**[8] | **Irene Terrenato MD**[9] | **Oreste Iocca MD**[10] | **Aldo Venuti PhD**[2] | **Raul Pellini MD**[1]

[1]Department of Otolaryngology – Head and Neck Surgery, IRCCS Regina Elena National Cancer Institute, Istituti Fisioterapici Ospitalieri (IFO), Rome, Italy

[2]HPV-Unit, UOSD Tumor Immunology and Immunotherapy, IRCCS Regina Elena National Cancer Institute, Istituti Fisioterapici Ospitalieri (IFO), Rome, Italy

[3]Department of Biochemical Sciences A. Rossi Fanelli, Sapienza University of Rome, Rome, Italy

[4]Oncogenomic and Epigenetic Unit, IRCCS Regina Elena National Cancer Institute, Istituti Fisioterapici Ospitalieri (IFO), Rome, Italy

[5]Translational Oncologic Research, IRCCS Regina Elena National Cancer Institute, Istituti Fisioterapici Ospitalieri (IFO), Rome, Italy

[6]Department of Pathology, IRCCS Regina Elena National Cancer Institute, Rome, Italy

[7]Department of Microbiology and Virology, IRCCS San Gallicano Dermatological Institute, Istituti Fisioterapici Ospitalieri (IFO), Rome, Italy

[8]Department of Radiology and Diagnostic Imaging, IRCCS Regina Elena National Cancer Institute, Istituti Fisioterapici Ospitalieri (IFO), Rome, Italy

[9]Clinical Trial Center – Biostatistics & Bioinformatics, IRCCS Regina Elena National Cancer Institute, Rome, Italy

[10]Division of Maxillofacial Surgery, Department of Surgical Science, University of Torino, Torino, Italy

**Correspondence**
Flaminia Campo, Department of Otolaryngology – Head and Neck Surgery, IRCCS Regina Elena National Cancer Institute, Via Elio Chianesi 53, 00144 Rome, Italy.
Email: flaminia.campo@ifo.it

**Funding information**
LILT 2020-21 Program; MAECI-Call for Joint Project Proposals Italy-Brazil, Grant/Award Number: BR22GR03

**Section Editor**: Patrick Ha

## Abstract

**Background:** First aim was to compare ddPCR assays of ctHPVDNA with p16 IHC and qualitative HPV PCR. Second aim was to carry out longitudinal blood sampling to test for association of ctHPVDNA with histological confirmed recurrence. Third aim was to perform a multidimensional assessment which included: (1) clinical features; (2) ctHPVDNA; (3) MRI-based tumor size measurements of primary tumor (PT) and cervical lymph node metastases (CLNM).

**Methods:** Plasma samples were collected before treatment and during follow-up, and ddPCR assay comprising E6 of HPV16 and HPV 33 and HPV 35 was used.

**Results:** Present study was conducted at diagnosis in 117 patients and revealed a ctHPVDNA sensitivity of 100% (95% CI 95.5–100) and a specificity

Flaminia Campo and Francesca Paolini contributed equally to this study.

of 94.4 (95% CI 81.3–99.3), positive predictive value (PPV) of 94.4 (95% CI 81.3–99.3), and negative predictive value (NPP) of 100% (95% CI 89.7–100). During follow-up ctHPVDNA had a sensitivity of 100% (95% CI 72.1–100)% and specificity of 98.4% (95% CI 91.7–100)%, PPV% of 90.9% (95% CI 62.3–98.4) and NPV % of 100% (95% CI 94.3–100) for ability to detect recurrence. Correlation between both the CLNM volume and the sum of PT and CLNM volume was observed.

**Conclusions:** ctHPVDNA was superior to p16 in identification of HPV-OPSCC at diagnosis. Introduction of ctHPVDNA, beyond diagnostic setting, represents a great opportunity to improve follow-up protocol of OPSCC patients.

**KEYWORDS**

circulating tumor DNA, human papillomavirus, liquid biopsy, MRI, oropharyngeal squamous cell carcinoma

## 1 | INTRODUCTION

The morbidity associated with treatment for oropharyngeal squamous cell carcinoma (OPSCC) and the favorable long-term survival when disease is detected and treated at early stage demonstrate the need to develop better prognostic/predictive biomarkers to optimize strategy for diagnosis and follow-up.[1–3]

Currently, National Comprehensive Cancer Network (NCCN) guidelines recommend use of tissue biopsy for detection of p16 immunohistochemistry (IHC) for the diagnosis of HPV + OPSCC.

However, up to 20% of patients who have p16 positive tumors test negative for HPV DNA or RNA.[4–6]

Double positivity, with tumors being positive for both p16 and HPV, has shown a better prognosis compared to a single marker of positivity and has shown to be correlated with the strongest diagnostic accuracy and prognostic value.[7] This highlights the importance of HPV evaluation and double positivity.[5,8]

Circulating tumor HPV DNA (ctHPVDNA) is emerging as promising biomarker for diagnosis and follow-up in patients with HPV-related OPSCC.[9,10] Interestingly, EBV circulating DNA load is currently considered in NCCN guidelines as new biomarker that reflects prognosis and change in response to nasopharyngeal cancer treatment. Thus, it is reasonable that ctHPVDNA could have the same diagnostic/prognostic impact/efficacy.

A large number of studies have evaluated the diagnostic value of ctHPVDNA by comparing the circulating biomarker with p16 IHC.[11–14] On the contrary, our group compared the ctHPVDNA with both the p16 and HPV testing.

There is a wide interpatient variability in ctHPVDNA level at diagnosis that seems related to tumor burden rather than other clinical pathological characteristics, but data is not fully consistent. For this reason, we utilized an improved magnetic resonance imaging (MRI)methodology to obtain a better evaluation of T and N volume by segmentation of the volume of interest (VOI) and lesion contour determination.

The first aim of this study was to establish droplet digital PCR (ddPCR) assays of plasma ctHPVDNA for multiple HPV strains and to compare these assays with conventional p16 IHC and qualitative HPV PCR of solid tumor biopsies for definition of HPV status at presentation.

The second aim was to carry out longitudinal blood sampling to test for association of ctHPVDNA with histological confirmed recurrence.

The third aim was to perform a multidimensional assessment of patients with OPSCC which included: (1) clinical features; (2) ctHPVDNA; (3) MRI-based tumor size measurements of both primary tutor (PT) and cervical lymph node metastases (CLNM).

## 2 | MATERIALS AND METHODS

### 2.1 | Study design and enrolment

All enrolled patients signed an informed consent to the protocol approved by the IRCCS Regina Elena National Cancer Institute, Istituti Fisioterapici Ospitalieri, Institutional Review Board (RS1647/22).

Eligibility criteria for inclusion were: (a) age older than 18 years; (b) willing to contribute blood samples for research purposes and provide informed consent. Patients with suspected OPSCC diagnosis were

prospectively identified upon presentation to the head and neck surgical oncology clinic at IRCCS Regina Elena National Cancer Institute, and consecutively enrolled. All patients underwent standard of care diagnostic workup and follow-up. All patients underwent histomorphology assessment, p16 IHC (CINtec® Histology kit, Roche Diagnostics) and direct HPV testing (Inno-LiPA HPV Genotyping Extra II, Fujirebio) on biopsy. This assay detects the DNA of 32 different mucosal HPVs, including those defined as "high-risk" by IARC (i.e., 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, and 59).

All patients underwent MRI or computed tomography scan and PET-CT at first diagnosis.

Pre-treatment blood samples were collected from all patients on recruitment. Sequential post-treatment blood samples were also collected from each patient at follow-up appointments, routinely scheduled as every 3 months for 2 years, followed by every 6 months for 2 years with additional appointments as clinically indicated.

## 2.2 | MRI-based tumor size measurements

MRI was performed using 1.5 T (Optima MR 450w, GE Healthcare, Milwaukee, WI) and 3 T (Discovery MR 750w, GE Healthcare, Milwaukee, WI) scan systems.

The primary tumor (PT) and each cervical lymph node metastasis (CLNM) were manually delineated, slice by slice, by two HN radiologists in consensus on axial T2-weighted fast spin-echo images (field of view, 26–28 cm; acquisition matrix, 256 × 256; slice thickness, 3–4 mm).

The 3D Slicer Software (Version 4.11) was used to delineate and quantify the volume of PT and CLNM.[15] An example of the lesion segmentation is shown in Figure 1.

In patients with several CLNMs, the total volume of CLNMs was obtained by summing each CLNM volume and this measurement was used for the subsequent statistical analyses.

## 2.3 | Blood collection, processing, and ddPCR

Five milliliters of whole blood were collected from each patient into BD Vacutainer K2 EDTA tubes (BD Biosciences, San Jose, CA) and refrigerated double-spun plasma (3000 and 13 000× rpm) was harvested within 1 h for storage at −80°C in cryotubes (Merck, Darmstadt, Germany). For ctHPVDNA analysis samples were thawed and cell free DNA was extracted from 1 mL of plasma using QIAamp MiniElute ccfDNA (Qiagen,

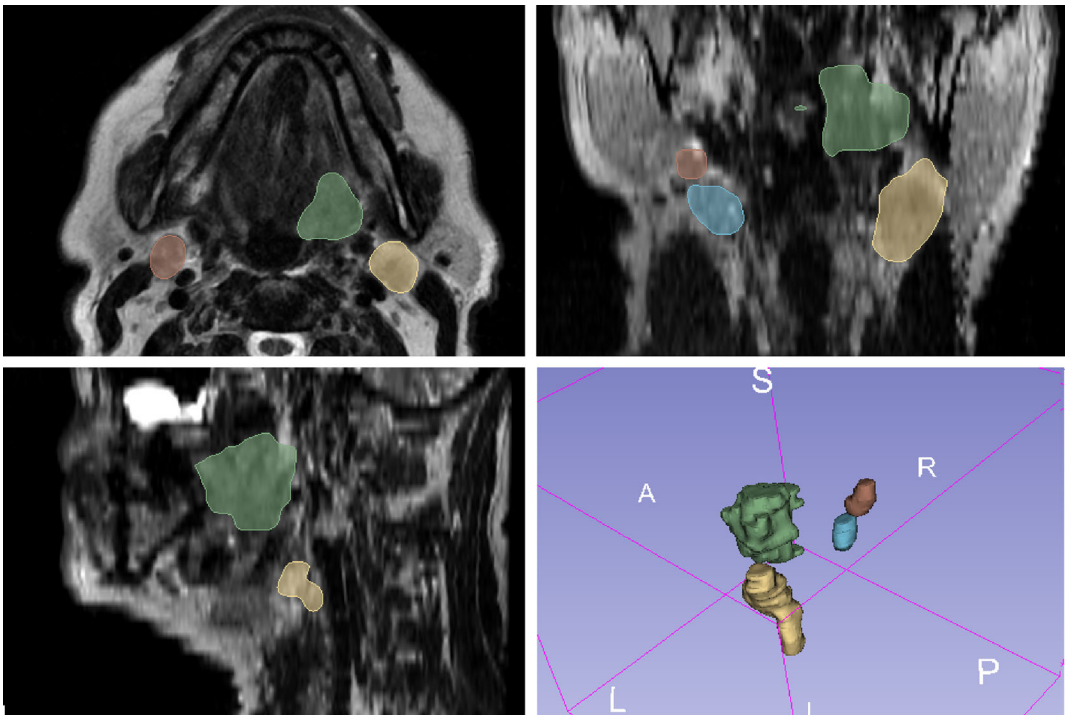


FIGURE 1 A 52-year-old man affected by an oropharyngeal squamous cell carcinoma of the left palatine tonsil with bilateral metastatic lymph nodes, shown on T2-weighted images in the axial (A), coronal (B), and sagittal (C) plane, with a 3D representation of the user-defined lesion volumes (D). [Color figure can be viewed at wileyonlinelibrary.com]

Germantown, MD) and recovered into a 60-μL final elution volume. Droplet digital PCR reagents 2x ddPCR Supermix for Probes (No dUTP), primers/probe to E6 gene for HPV subtypes 16, 33, and 35 and ctDNA were used in 22 μL reaction mixture. The Primers and FAM-ZEN dual quencher probes were synthesized by Integrated DNA Technology (IDT) according to Chera et al.[16] Droplet generation and transfer of emulsified samples to PCR plates was performed according to manufacturer's instructions (Instruction Manual, QX200™ Droplet Generator, Bio-Rad). The cycling protocol was: 95°C enzyme activation step for 5 min followed by 40 cycles of a two-step cycling protocol (95°C for 30 s and 60°C for 1 min). The ramp rate between these steps was slowed to 2°C/s.

The assay threshold sensitivity was set at three standard deviations above the background observed in no template (nuclease-free water) controls. Using serial dilution of HPV16, 33 and 35 DNA, analytical sensitivity and precision was determined to be $2 \pm 1.1$ copies per reaction.

The absolute quantity of ctDNA per sample (copies/μL) was processed using QuantaSoft (v.1.7.4).

## 2.4 | Statistical analysis

Descriptive statistics were calculated for all variables of interest. Categorical variables were summarized with frequencies and percentage values while continuous variables with median and range. Concordance between ctHPVDNA and p16/HPV-DNA, considered as gold standard, was calculated with un-weighted Cohen's Kappa. Sensitivity, specificity, positive predicted values, and negative predictive values with their relative 95% confidence interval (95% CI) were also calculated. Comparisons between ctHPVDNA and socio-demographic data such as smoking and alcohol intake were assessed with Pearson's chi-square test or Mann–Whitney nonparametric test, when appropriate. Correlation between ctHPVDNA and imaging data was assessed with Spearman's Rho coefficient. A $p$-value $<0.05$ was considered statistically significant. All statistical analysis was carried out with SPSS v. 29.0.

# 3 | RESULTS

## 3.1 | Patient characteristics

From January 2022 to April 2024, 117 consecutive patients were prospectively enrolled in the study.

Overall, 92 patients were OPSCC p16+, 25 patients were OPSCC p16−. In the study, 69 patients were p16+/HPV16+, 3 were p16/HPV33+, 6 were p16/HPV35+, 12 were p16+/HPV−, 24 were p16−/HPV−, and 1 was p16−/HPV16+.

Clinical and pathological features were collected for each patient, including age, sex, smoking status, alcohol intake status, tumor site, and clinical TNM staging (AJCC 8th edition) (Table 1).

Forty-five patients were evaluated with MRI at diagnosis, remaining patients with computer tomography scan.

**TABLE 1** Patient's characteristics.

| Characteristics | *N* (%) |
|---|---|
| Sex | |
| Male | 86 (73.5) |
| Female | 31 (26.5) |
| Age at diagnosis | |
| Median [min–max] | 62 [45–93] |
| p16 | |
| Pos | 92 (78.6) |
| Neg | 25 (21.4) |
| HPV-DNA test | |
| Pos | 81 (69.2) |
| Neg | 36 (30.8) |
| cENE | |
| Pos | 57 (48.7) |
| Neg | 60 (51.3) |
| T size | |
| T0 | 13 (11.1) |
| T1 + T2 | 45 (38.4) |
| T3 + T4 | 59 (50.5) |
| Lymphnode status | |
| N0 | 18 (15.4) |
| N1 | 60 (51.3) |
| N2 + N3 | 39 (33.3) |
| Diagnosis | |
| Base of tongue | 51 (43.9) |
| Tonsil | 49 (41.9) |
| Palate | 4 (3.4) |
| Unknown site | 13 (10.8) |
| Treatment modality | |
| Chemoradiation | 57 (48.7) |
| Radiation | 8 (6.8) |
| Surgery | 23 (19.6) |
| Surgery and adjuvant chemoradiotherapy | 16 (13.6) |
| Surgery and adjuvant radiotherapy | 13 (11.3) |

### 3.2 | ctHPVDNA at diagnosis

Plasma samples at diagnosis were analyzed on 117 patients. We detected ctHPVDNA in 83 patients: 81 were HPV+ and 2 HPV−. This revealed a sensitivity of 100% (95% CI 95.5–100) and a specificity of 94.4 (95% CI 81.3–99.3), positive predictive value (PPV) of 94.4 (95% CI 81.3–99.3), and negative predictive value (NPP) of 100% (95% CI 89.7–100).

p16 was detected in 92 patients: 80 were HPV+ and 12 were HPV−. This revealed a sensitivity of 98.8% (95% CI 93.3–99.7) and a specificity of 66.7% (95% CI 49–81.5), PPV of 86.9% (95% CI 80.7–91.3), and NPP of 96% (95% CI 77.1–99.4).

Cohen's Kappa indicated that there was a substantial concordance between p16 and HPV DNA $K = 0.72$ (95% CI 57–85) (Table S1, Supporting Information).

Cohen's Kappa between ctHPVDNA and p16 was 0.71 (95% CI 0.56–0.73) indicating substantial concordance, whereas an almost perfect concordance of 96 (95% CI 90–10) between ctHPVDNA and HPV DNA was detected. This result was also confirmed when we categorized patients as double positive p16/HPV DNA with a value of *k* 94 (95% CI 87–100) (Table 2).

### 3.3 | Longitudinal monitoring of ctHPVDNA

To test for correlation of treatment response with ctHPVDNA, we collected sequential post-treatment blood samples in 60 of the 82 patients with detectable ctHPVDNA pre- treatment and in 14 of the 35 patients with undetectable ctHPVDNA pre-treatment.

All 14 patients with undetectable ctHPVDNA pre-treatment displayed negative results on all remaining samples.

All 60 patients with detectable ctHPVDNA pre-treatment in whom we collected sequential post-treatment blood samples underwent treatment with curative intent. Forty-nine patients reached undetectable HPV levels average at 10 week post-treatment and remained undetectable for the remainder of the study. None of these patients developed a recurrence.

Eleven patients demonstrated either a persistently elevated or a subsequent increase in their HPV copy number after initially becoming negative post-treatment. Seven of these patients never reached undetectable HPV levels post-treatment. In six patients was observed regional disease progression and in one patient distant disease progression. In five patients, ctHPVDNA copy number fell to zero post-treatment and subsequently increased. In four of these patients, the rise in HPV copy-number either pre-dated or correlated with detection of progressive regional or metastatic disease. In the final patient, the rise in ctHPVDNA was detected at 9 months post-treatment and ctHPVDNA level continued to increase until the last available blood sample with no clinical or radiological evidence of disease progression ant 22 months post-treatment. Thus, overall in this cohort, ctHPVDNA testing had a sensitivity of 100% (95% CI 72.1–100)% and specificity of 98.4% (95% CI 91.7–100)%, PPV% of 90.9% (95% CI 62.3–98.4), and NPV % of 100% (95% CI 94.3–100) for ability to detect recurrence.

### 3.4 | ctHPVDNA copy correlation with clinical pathological features

No trend of increasing ctHPVDNA copies was observed for age and sex.

We did not observe a trend of increasing ctHPVDNA copies at baseline in relationship with increasing N-category, or T-category.

MRI-based measurement of tumor size did not show an association between ctHPVDNA copies per plasma ml and PT volume at baseline, while positive significant relationships were found with both the CLNM volume (Rho = 0.42, $p = 0.004$) and the summa of PT and CLNM volume (Rho = 0.51, $p < 0.001$) (Table S2). MRI of two

**TABLE 2** Agreement of ctHPVDNA versus p16, HPVDNA and p16+/HPVDNA+.

| | | p16 | | | HPVDNA | | | HPVDNA+ p16+ | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | p16 (−) | p16 (+) | Cohen's *K* 95% CI | HPV DNA (−) | HPVDNA (+) | Cohen's *K* 95% CI | No | Yes | Cohen's *K* (95% CI) |
| ctHPVDNA | ctHPVDNA (−) | 23 | 11 | 0.71 (0.56–0.73) | 34 | 0 | 0.96 (0.9–1) | 34 | 0 | 0.94 (0.87–1) |
| | ctHPVDNA (+) | 2 | 81 | | 2 | 81 | | 3 | 80 | |
| Total | | 25 | 92 | | 36 | 81 | | 37 | 80 | |

patients with high and low copies of ctHPVDNA, respectively, are showed in Figure 2.

## 4 | DISCUSSION

Present study was conducted at diagnosis in 117 patients and revealed a ctHPVDNA sensitivity of 100% (95% CI 95.5–100) and a specificity of 94.4 (95% CI 81.3–99.3), positive predictive value (PPV) of 94.4 (95% CI 81.3–99.3) and negative predictive value (NPP) of 100% (95% CI 89.7–100).

The high sensitivity and specificity of ctHPVDNA in our study are consistent with a recent meta-analysis that explored the accuracy of ctHPVDNA by ddPCR in patients at diagnosis of HPV + OPSCC. This meta-analysis combined outcomes from 729 p16+ OPSCC patients and 269 p16- controls. The meta-analytic study estimated the diagnostic performance of ctHPVDNA as follows: pooled sensitivity and specificity of 0.90 (95% CI: 0.82–0.94) and 0.94 (95% CI: 0, 85–0.98), respectively.[14]

However, our study was performed comparing the ctHPVDNA with both the p16 and HPV testing. It has been shown that up to 20% of patients with p16+ tumors test negative for HPV.[5,6,8] In our cohort of patients, 13.04% (12/92 patients) were p16+ but HPV negative. Therefore, in the era of precision medicine the use of p16 alone might not be appropriate in clinical practice and our data on ctHPVDNA confirm this assumption.[5]

Data clear indicates that during diagnostic workup, ctHPVDNA is better that p16 IHC in order to identify patients with HPV-related OPSCC. Furthermore, our results demonstrate that concordance between p16+/HPV DNA test and ctHPVDNA is excellent; this suggests

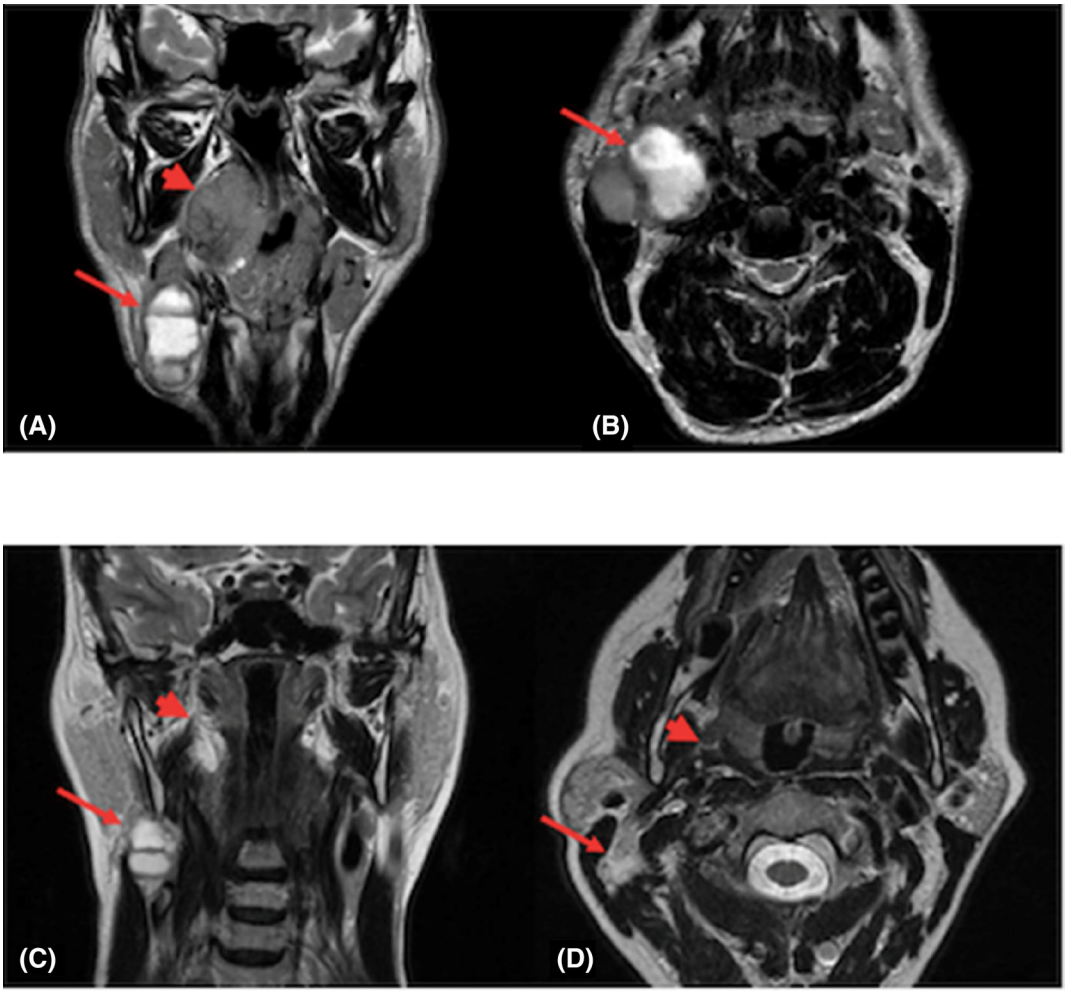


FIGURE 2 MRI T2-weighted sequences, in axial (B, D) and coronal (A, C) planes showing two HPV+ OPSCC of different size in the right palatine tonsil (arrowhead), with a partially metastatic necrotic lymph node in the right IIa level (arrow). Patient number 34: copies of HPV per plasma ml were 8989.8 with voluminous primary tumor and large metastatic lymph nodes (A, B). Patient number 3: copies of HPV per plasma ml were 71.2 with small primary tumor and metastatic lymph nodes (C, D). [Color figure can be viewed at wileyonlinelibrary.com]

that liquid biopsy could be useful in identifying the subgroup of patients who have a better oncological outcome. In agreement with Jakobsen's data,[17] ctHPVDNA may be useful to identify patients suitable for de-escalation therapy.

Regarding follow-up of OPSCC, several critical issues have emerged regarding this surveillance modality. For instance, it has been highlighted that the use of positron emission tomography (PET) scans in post-radio chemotherapy treatment is characterized by a high number of false positives.[18–20] PET-CTs have a poor positive predictive value of 30% on 12 week surveillance for HPV-OPSCC.[21] A recent meta-analysis highlighted that PET-CT results were equivocal for 22.5% (95% CI, 12.5–36.9) and equivocal/positive for 34.2% of patients (95% CI, 25.1–44.5).[22] Recently, researchers and clinicians have begun to evaluate the clinical utility of ctHPVDNA and TTMV-HPVDNA in biological fluids for the diagnosis and monitoring of patients with HPV-positive cancers.[17,23–27] Tumor progression is associated with the expression of oncogenic viral DNA and proteins. Current study confirms data present in the literature. Thus, overall in this cohort, ctHPVDNA testing had a sensitivity of 100% (95% CI 72.1–100)% and specificity of 98.4% (95% CI 91.7–100)%, PPV% of 90.9% (95% CI 62.3–98.4), NPV% of 100% (95% CI 94.3–100), and accuracy of 98.6% (95% CI 92.7–99.7) for ability to detect recurrence. At the moment we cannot establish why ctHPVDNA was persistently elevated in one patient with no evidence of disease. However, we will continue to monitor the patient over time.

Many research groups have tried to correlate ctHPVDNA with other clinical features of OPSCC.

Conflicting reports have linked high level of ctHPVDNA with T stage and N stage.[16,28] However, we did not find that higher baseline levels of ctHPVDNA were related to increasing T category or N category.

Our study demonstrated correlation between both the CLNM volume (Rho = 0.42, $p = 0.004$) and the sum of PT and CLNM volume (Rho = 0.51, $p < 0.001$), but highlighted that only a highly precise measurement such as the segmentation of the volume of interest (VOI) allowed to correlate ctHPVDNA copy number with tumor burden.

Principal limitation of the study is the number of enrolled patients, but it was statistically appropriate to ascertain differences among groups. Actually, available literature reports were obtained on OPSCC patient cohort of similar magnitude of our study. In addition, the ddPCR assay was for the detection of only three HPV strains (HPV16, 33, and 35).

In summary ctHPVDNA was superior to p16 IHC in the diagnostic identification of HPV related OPSCC. Our data, in agreement with literature data, allow us to suggest an improvement in the diagnostic workup through the introduction of ctHPVDNA.

Finally, introduction of ctHPVDNA, beyond diagnostic setting, represents a great opportunity to improve follow-up protocol of OPSCC patients.

In the future, a multidimensional integrated approach which links multiple clinical, radiological, and laboratory data will contribute to obtain the best follow-up strategies for the follow-up of HPV-OPSCC.

## ACKNOWLEDGMENTS

A.V. and F.P. was partially supported by LILT 2020-21 Program and MAECI-Call for Joint Project Proposals Italy-Brazil # BR22GR03. Open access funding provided by BIBLIOSAN.

## CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

## DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

## ORCID

*Flaminia Campo* https://orcid.org/0000-0002-9249-7573

## REFERENCES

1. Campo F, Iocca O, De Virgilio A, et al. Treatment of oropharyngeal squamous cell carcinoma: Is swallowing quality better after TORS or RT? *Radiother Oncol*. 2023;183:109547.
2. Mehanna H, Gaunt P, Kong A, et al. CompARE: study protocol for a phase III randomised controlled platform trial comparing alternative regimens for escalating treatment of intermediate and high-risk oropharyngeal cancer. *Trials*. 2024;25(1):50.
3. De Virgilio A, Iocca O, Malvezzi L, et al. The emerging role of robotic surgery among minimally invasive surgical approaches in the treatment of hypopharyngeal carcinoma: systematic review and meta-analysis. *J Clin Med*. 2019;8(2):256.
4. Hoffmann M, Tribius S, Quabius ES, et al. HPV DNA, E6*I-mRNA expression and p16INK4A immunohistochemistry in head and neck cancer—how valid is p16INK4A as surrogate marker? *Cancer Lett*. 2012;323(1):88-96.
5. Mehanna H, Taberna M, von Buchwald C, et al. Prognostic implications of p16 and HPV discordance in oropharyngeal cancer (HNCIG-EPIC-OPC): a multicentre, multinational, individual patient data analysis. *Lancet Oncol*. 2023;24(3):239-251.
6. Craig SG, Anderson LA, Schache AG, et al. Recommendations for determining HPV status in patients with oropharyngeal cancers under TNM8 guidelines: a two-tier approach. *Br J Cancer*. 2019;120(8):827-833.
7. Mehanna H, Alemany L, von Buchwald C. Advances in testing for human papillomavirus-mediated head and neck cancer. *Curr Opin Oncol*. 2024;36(3):143-146.
8. Mena M, Taberna M, Tous S, et al. Double positivity for HPV-DNA/p16(ink4a) is the biomarker with strongest diagnostic

accuracy and prognostic value for human papillomavirus related oropharyngeal cancer patients. *Oral Oncol*. 2018;78:137-144.
9. Krasniqi E, Barba M, Venuti A, et al. Circulating HPV DNA in the management of oropharyngeal and cervical cancers: current knowledge and future perspectives. *J Clin Med*. 2021;10(7):1525.
10. Lang Kuhs KA, Brenner JC, Holsinger FC, Rettig EM. Circulating tumor HPV DNA for surveillance of HPV-positive oropharyngeal squamous cell carcinoma: a narrative review. *JAMA Oncol*. 2023;9(12):1716-1724.
11. Tanaka H, Suzuki M, Takemoto N, et al. Performance of oral HPV DNA, oral HPV mRNA and circulating tumor HPV DNA in the detection of HPV-related oropharyngeal cancer and cancer of unknown primary. *Int J Cancer*. 2022;150(1):174-186.
12. Akashi K, Sakai T, Fukuoka O, et al. Usefulness of circulating tumor DNA by targeting human papilloma virus-derived sequences as a biomarker in p16-positive oropharyngeal cancer. *Sci Rep*. 2022;12(1):572.
13. Faden DL. Liquid biopsy for the diagnosis of HPV-associated head and neck cancer. *Cancer Cytopathol*. 2022;130(1):12-15.
14. Paolini F, Campo F, Iocca O, et al. It is time to improve the diagnostic workup of oropharyngeal cancer with circulating tumor HPV DNA: systematic review and meta-analysis. *Head Neck*. 2023;45:2945-2954.
15. Fedorov A, Beichel R, Kalpathy-Cramer J, et al. 3D slicer as an image computing platform for the quantitative imaging network. *Magn Reson Imaging*. 2012;30(9):1323-1341.
16. Chera BS, Kumar S, Beaty BT, et al. Rapid clearance profile of plasma circulating tumor HPV type 16 DNA during chemoradiotherapy correlates with disease control in HPV-associated oropharyngeal cancer. *Clin Cancer Res*. 2019;25(15):4682-4690.
17. Jakobsen KK, Bendtsen SK, Pallisgaard N, et al. Liquid biopsies with circulating plasma HPV-DNA measurements—a clinically applicable surveillance tool for patients with HPV-positive oropharyngeal cancer. *Clin Cancer Res*. 2023;29(19):3914-3923.
18. Corpman DW, Masroor F, Carpenter DM, Nayak S, Gurushanthaiah D, Wang KH. Posttreatment surveillance PET/CT for HPV-associated oropharyngeal cancer. *Head Neck*. 2019;41(2):456-462.
19. Rulach R, Zhou S, Hendry F, et al. 12 week PET-CT has low positive predictive value for nodal residual disease in human papillomavirus-positive oropharyngeal cancers. *Oral Oncol*. 2019;97:76-81.
20. Yu Y, Mabray M, Silveira W, et al. Earlier and more specific detection of persistent neck disease with diffusion-weighted MRI versus subsequent PET/CT after definitive chemoradiation for oropharyngeal squamous cell carcinoma. *Head Neck*. 2017;39(3):432-438.
21. Zhou S, Chan C, Rulach R, et al. Long term survival in patients with human papillomavirus-positive oropharyngeal cancer and equivocal response on 12-week PET-CT is not compromised by the omission of neck dissection. *Oral Oncol*. 2022;128:105870.
22. Mathews F, Irizarry R, Rosenfeld R, Sundaram K. Systematic review and meta-analysis of post-treatment PET/CT in HPV-associated oropharyngeal cancer. *Ann Otol Rhinol Laryngol*. 2022;131(6):595-603.
23. 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.
24. Lalondrelle S, Lee J, Cutts RJ, et al. Predicting response to radical chemoradiotherapy with circulating HPV DNA (cHPV-DNA) in locally advanced uterine cervix cancer. *Cancers (Basel)*. 2023;15(5):1387.
25. Li LQ, Adamowicz M, Wescott RA, et al. The role of liquid biopsy in management of the neck with indeterminate response on post-treatment imaging following non-surgical management of oropharyngeal cancer. *Eur J Surg Oncol*. 2023;49(1):55-59.
26. Campo F, Zocchi J, Moretto S, et al. Cell-free human papillomavirus-DNA for monitoring treatment response of head and neck squamous cell carcinoma: systematic review and meta-analysis. *Laryngoscope*. 2021;132:560-568.
27. Edwards DM, Gharzai LA, Wang W, et al. Follow-up for human papillomavirus-related oropharynx cancer concentrated on unequal symptom change (FOCUS): prospective patient-reported outcome collection. *Head Neck*. 2024. doi:10.1002/hed.27778. Online ahead of print.
28. Siravegna G, O'Boyle CJ, Varmeh S, et al. Cell-free HPV DNA provides an accurate and rapid diagnosis of HPV-associated head and neck cancer. *Clin Cancer Res*. 2022;28(4):719-727.

## SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

**How to cite this article:** Campo F, Paolini F, Manciocco V, et al. Circulating tumor HPV DNA in the management of HPV+ oropharyngeal cancer and its correlation with MRI. *Head & Neck*. 2024;46(9):2206-2213. doi:10.1002/hed.27866