**Performance of ddPCR and qPCR to detect cell-free HPV16 DNA in liquid biopsies from patients with HPV-related oropharyngeal squamous cell carcinoma at diagnosis and recurrence.**

Imke Demers[1]
Nora Wuerdemann[2], Christina Alidousty[3], Rishabh Jain[4], Oliver Siefer[5], Christian Huebbers[6], Julie George[4], Steffi Silling[7], Ulrike Wieland[7], Baki Akgül[8], Barbara Holz[4], Kathrin Moellenhoff[4], Shachi Jenny Sharma[4], Darius Schafigh[9], Marc Schlamann[9], Claus Wittekindt[10], Reinhard Büttner[4], Alexander Quaas[4], Steffen Wagner[11], Anne Schultheis[3], Ernst-Jan Speel[1] and Jens Peter Klussmann[5]
[1] Department of Pathology, Maastricht University Medical Centre
[2] Department of Otorhinolaryngology, University of Cologne
[3] Institute of Pathology, University of Cologne
[4] University of Cologne
[5] University of Cologne, Center for Molecular Medicine Cologne (CMMC)
[6] University of Colgone, Center for Molecular Medicine Cologne (CMMC)
[7] Institute of Virology, University of Cologne
[8] Institute of Virology
[9] Department of Radiology, University of Cologne
[10] University of Dortmund
[11] University of Giessen

## Background & objectives

**Background:** Globally the incidences of oropharyngeal squamous cell carcinoma (OPSCC), especially those associated with high-risk human papillomavirus (HPV; predominantly HPV type 16), are rising. Although patients with HPV-related OPSCC present with better survival, up to 25% develop recurrent or distant metastatic disease. Biomarkers to monitor therapy response, minimal residual disease and tumor burden during follow-up are urgently needed. The detection of cell-free (cf)HPV-DNA in blood plasma offers the opportunity for minimally invasive disease monitoring and early recurrence detection.

**Objective:** We aim to perform a retrospective analysis to determine the clinical utility of cfHPV16-DNA by designing, validating, and comparing the performance of qPCR and ddPCR assays, obtaining real-time information of tumor-associated changes in HPV-related OPSCC patients.

## Methods

**Methods:** We examined 33 patients with HPV-related OPSCC and 21 controls (healthy donors and HPV-negative OPSCC patients), from 2019 to 2022, to investigate cfHPV16-DNA status prior to and during therapy as well as throughout follow-up (every 3-4 months, up to 4 years).

qPCR and ddPCR were applied to quantify the concentration of cfHPV16-DNA in plasma, using the viral gene E6 as a target in both methods.

## Results

**Results:** No cfHPV16-DNA or few copies (<10 copies/ml plasma) were detected in healthy donors or HPV-negative OPSCC patients. Plasma samples from patients with HPV-related OPSCC collected prior to treatment were positive for cfHPV16-DNA. At the cut-off of 10 copies/ml plasma, ddPCR showed 79% sensitivity, 80% specificity, 70% NPV, and 90% PPV, whereas qPCR showed 73% sensitivity, 80% specificity, 60% NPV, 90% PPV. Patients without clinical evidence of recurrence had significantly lower cfHPV16-DNA concentrations after therapy, whereas increase of copy number was seen in patients with recurrent disease.

## Conclusion

**Conclusion:** Our results demonstrate that ddPCR is a more sensitive approach than qPCR to detect cfHPV16-DNA in plasma of patients with HPV16-related OPSCC and therefore presents a promising diagnostic tool. Nevertheless, prospective studies with expanded patient cohorts and close monitoring are necessary to investigate the kinetics of cfHPV16-DNA in relation to therapy response and the development of recurrent and metastatic disease. Furthermore, extension of the assays to allow detection of other high-risk HPV types that may occur in HPV-positive OPSCC is warranted.