Application of Plasma Circulating HPV DNA Testing to Management of Cervical Intraepithelial Neoplasia

Vaishnavi Siripurapu, Shivani Sud, MD, Lisa Rahangdale, MD, Becky Green, MSw, Jenna Nakagawa, MD, Caroline Cochrane, MD, Ashley Weiner, MD, PhD

ABSTRACT

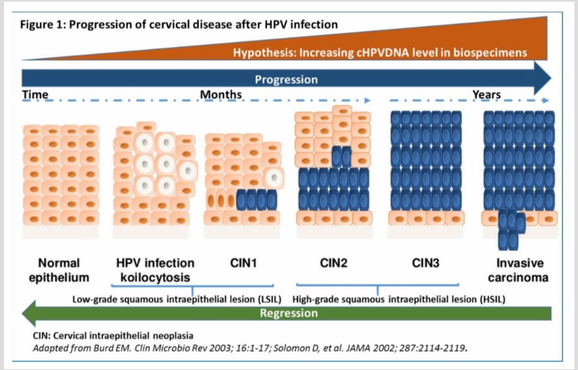
The human papilloma virus (HPV) causes several cancers. In patients with HPV-related cancer, HPV DNA has been shown to circulate in the patient's bloodstream (cHPVDNA). The goal of this project is to understand the use of cHPVDNA as a blood-based biomarker for Cervical Intraepithelial Neoplasia (CIN) and elucidate cHPVDNA's effectiveness for CIN detection. Cervical cancer develops in a stepwise fashion through CIN 1,2,3 with CIN 1 lesions being the least likely to progress to cancer and CIN 2-3 lesions being more precancerous, heightening the importance of early detection. cHPVDNA has been detected in the plasma of patients with CIN, proving potential as a biomarker. In the future, we hope to develop a blood-based test for cervical precancer and improve women's access to cancer screenings.

CONTACT

Vaishnavi Siripurapu
UNC-Chapel Hill
Email: Vaish16@ad.unc.edu
Phone: 9195275545

INTRODUCTION

Cervical cancer is a disease that impacts thousands of women every year. There an estimated 528,000 new cases and 266,000 deaths annually, making cervical cancer the second-most common reproductive cancer. Screening programs have dramatically reduced the morbidity of cervical cancer as disease detected at the early CIN 1 stage has a significant chance of regressing to normal. A PAP Smear is the recommended screening test for cervical cancer. However, PAP smears are invasive and can cause discomfort to patients. It is imperative to explore alternative methods of screening for cervical cancer due to its prevalence, treatability, and invasiveness of current screening procedures.



Recent work from the Gupta lab has shown that cHPVDNA has the potential to be an effective biomarker for invasive disease. In a study of 115 control subjects comprising of healthy and non-HPV based cancer patients as well as 103 HPV-related tonsil cancer patients, optimized cHPVDNA assays had a had 98% specificity and 89% sensitivity. These results indicate that cHPVDNA has the ability to indicate invasive HPV-related disease, and could be applicable to CIN.

METHODS: Recruitment

To begin work, we will enroll 3 cohorts of patients. These cohorts will consist of:
25 patients who are HR-HPV negative as a control
30 patients with CIN1
30 patients with CIN2-3.

CIN patients will be recruited from the UNC dysplasia clinic and minor procedure clinic and HR-HPV negative patients will be recruited from UNC Gynecology Clinics. Patients will be recruited via direct clinic recruitment, phone call inquiries, and email contact. Patients who enroll will be assigned a participant ID and their biospecimens will be collected (as clinically indicated).

For participation, patients will be offered gift cards and parking vouchers.

We aim to enroll four patients per week and complete PCR on half of patients within two months in order to begin baseline data analysis.

cHPVDNA will be collected at the time of biopsy. Patients receiving LEEPs will then be followed-up in clinic within 2-4 weeks for repeat blood draw for cHPVDNA. Cervical swabs will be taken for all cohorts and will serve as a control for our assay.

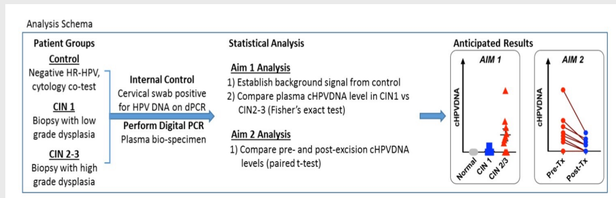
METHODS: Specimen Analysis

After the samples have been collected in the first months, specimen analysis will begin and continue throughout the duration of the project.

We will use 5' hydrolysis probes as well as primers which are specifically designed to detect an amplicon of around 75 base pairs within the HPV E7 gene.

A digital droplet PCR assay will be performed on this mixture using the QX100 and QX200 platforms. The results of each of these assays will be validated against control DNA samples, and these assays have already been shown to be specific for our strain of interest.

For statistical analysis, the samples will be analyzed using standardized thresholds. These thresholds will correlate to positive and negative droplets. Sample quality will be assessed using a dPCR assay for a control locus on chromosome 6 using QuantaSoft software.



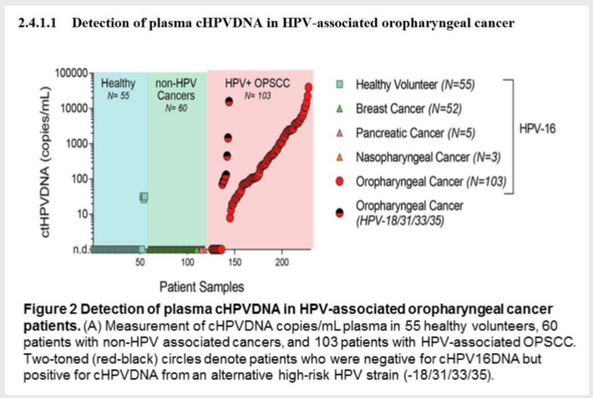
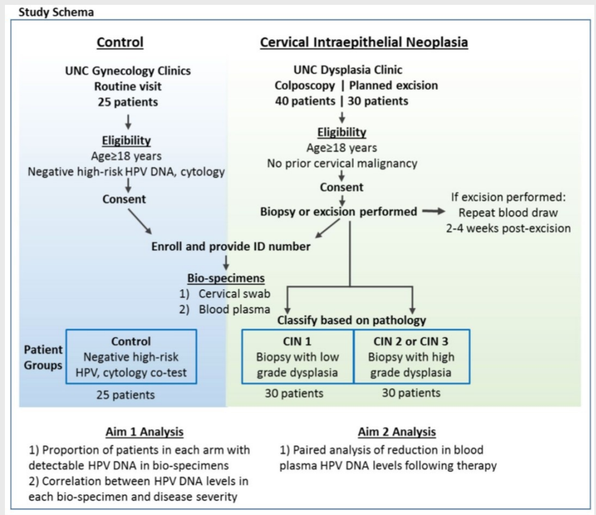


Figure 2 Detection of plasma cHPVDNA in HPV-associated oropharyngeal cancer patients. (A) Measurement of cHPVDNA copies/mL plasma in 55 healthy volunteers, 60 patients with non-HPV associated cancers, and 103 patients with HPV-associated OPSCC. Two-toned (red-black) circles denote patients who were negative for cHPV16DNA but positive for cHPVDNA from an alternative high-risk HPV strain (-18/31/33/35).



RESULTS

The tables below indicate our results in terms of patient recruitment and sample procurement.

Category	Target	Current	Remaining
Control	25	15	10
CIN 1	30	17	13
CIN 2-3	30	27	3

We have 10 remaining patients to reach our control total, 13 remaining patients to reach our CIN1 patients total, and 3 remaining patients to reach our CIN2-3 total. We also had 17 with abnormal screening unrelated to CIN, for a total of 76 patients in our current recruitment attempts. We will continue recruitment of these patients from UNC Clinics throughout 2021.

Follow-Up Samples:	18 Blood Samples Post-LEEP

In total we have received 18 total Blood Samples post LEEP for analysis. Unfortunately, COVID has severely impacted the timeline of this study

FUTURE DIRECTIONS

In the future, we hope to further propel the continuation of this project by reaching our patient recruitment and sample procurement targets in the Fall and Winter of 2020, completing sample analysis and data interpretation by Fall of 2021.

After data analysis, we will continue to contextualize this work in the area of past work in this field such as cHPVDNA circulated in head and neck cancer patients to further understand and propel the impacts of this research.

REFERENCES

Gupta, G. P. et al. Circulating Tumor HPV16 DNA as a Biomarker of Tumor Genomics and Disease Control in HPV-associated Oropharyngeal Squamous Cell Carcinoma. Int. J. Radiat. Oncol. 100, 1310–1311 (2018).

Sud, Shivani et al. 2019. "Application of plasma circulating HPV DNA testing to management of cervical intraepithelial neoplasia"

Torre, L. A. et al. Global cancer statistics, 2012. CA. Cancer J. Clin. 65, 87–108 (2015).

Ostör, A. G. Natural history of cervical intraepithelial neoplasia: a critical review. Int. J. Gynecol. Pathol. Off. J. Int. Soc. Gynecol. Pathol. 12, 186–192 (1993).