Screening for Cervical Intraepithelial Neoplasia Using Self-collected Menstrual Blood

Study Protocol and Statistical Analysis Plan

NCT03638427

March 17, 2020

TITLE: Screening for Cervical Intraepithelial Neoplasia Using Self-Collected Menstrual Blood

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Version 4 / Version Date (03-17-2020)

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1.0 SPECIFIC STUDY AIMS

Specific Aim #1: To compare the accuracy and percent analytic agreement of clinician- and self-collected cervico-vaginal brush samples with Q-pad-collected menstrual blood for HR-HPV detection in HR-HPV positive women and women with no previous history of HR-HPV.

Specific Aim #2: 2.1: To assess the ability to detect oncoproteins in Q-pad collected menstrual blood samples from women with ASCUS+ to determine the utility in cervical cancer/precancer detection and 2.2: to test the utility of a low and high threshold HR-HPV reflex scheme to similarly increase the specificity of HR-HPV testing derived from menstrual blood.

2.0 BACKGROUND AND RATIONALE

Cervical cancer (CaCx) is the fourth most common cancer among women worldwide, and the 2nd highest cause of cancer-related mortality among women[1]. Most cases of CaCx are preventable through the combination of screening and access to appropriate treatment of precancerous lesions[2]. Papanicolaou (Pap) cytology screening has been the most widely applied screening method for cervical cancer or precancer but has variable sensitivity and specificity and, globally, cytology is not effectively available to women in developing countries (where 80% or all cervical cancers occur)[3]. The combination of variable test qualities and limited access results in continued high incidence of fatal cancers in many settings.

In recent years, tests to detect Human Papilloma Virus (HPV), particularly high-risk subtypes (HR-HPV) from cervical samples have been introduced in many settings and are poised to become a primary screening approach in the future. A known limitation to DNA-based HR-HPV testing is that it can only discriminate between presence or absence of an HPV specific DNA and cannot determine whether presence of the virus indicates mere infection or pre-cancer/cancer. Several studies have found that the molecular switch for the development of Cervical Intra-epithelial Neoplasia (dysplasia or CIN) and ultimately CaCx is the expression of the E6 and E7 onco-proteins resulting from infection with oncogenic HPV[4]. Results demonstrates that the specificity of HR-HPV E6/E7 mRNA is higher than that of HR-HPV[5-6] and that HR-HPV E6-E7 mRNA measured at baseline predicts future CIN2 or CIN3 development[7]. Finally, it has been shown that compared with HR-HPV testing alone, HR-HPV/E6-E7 mRNA testing could reduce half of the colposcopy referrals generated by current prevention rubrics[4]. Alternatively, changing the cycle threshold for a positive HR_HPV test from low (high sensitivity) to high (high specificity) as a serial "reflex" test could have the same effect. In addition, the evolution to primary testing with HR-HPV has been accomplished in some settings using specimens obtained from patient self-collected swabs, obviating the need for the vast majority of women to undergo a gynecological exam, which is invasive, uncomfortable, and resource-intense.

Detection of HR-HPV with E6/E7 via self-collected samples could improve access to screening, improve sensitivity, and increase objectivity of testing. A variety of self-collection devices have been used to detect HPV including swabs, brushes, cervical

lavage devices, tampons, and urine.[8] While most of these devices have comparable sensitivity for HR-HPV (compared to clinician collected samples)[9], logistical challenges limit their utility. Most devices require instructions on proper insertion into the vagina (which can be culturally challenging in some settings), the specimens are difficult to transport, and often require storage in expensive, flammable fixative solution. A novel and innovative alternative involves the use of **menstrual blood** for the same diagnostic purpose. To this end, a specialized collection device integrated into an ordinary, inexpensive menstrual pad has been developed. Specifically, the "The Q-Pad" is a small, flexible, and easy-to-transport device involving dried paper – which essentially creates a Dried Blood Spot (DBS) specimen – embedded within a sanitary napkin. For purposes of CaCx screening, such a collection device does not require intra-vaginal manipulation of a brush/swab or lavage device and could improve participation in community screening efforts while also being easier to transport than urine or cervicovaginal samples. Menstrual blood has already been shown to corelate well with a number of commonly used serum tests such as Hemoglobin A1C and TSH[10].

Menstrual blood is composed of three distinct body fluids: whole blood, vaginal fluid, and endometrial cells shed from the endometrial lining. While menstrual blood has been presumed to be virtually identical to whole blood, important differences exist and have been understudied. Studies from the 1980s found that menstrual fluid lacks factor X, pro-thrombin, and free thrombin[15]. Proteomic and small-scale HPV detection studies have been recently published on menstrual blood[16], yet rigorous, larger-scale and implementation-level studies remain to be performed. In preliminary (unpublished) findings on HR-HPV screening with the Q-pad, we found that device detected HR-HPV using the GeneXpert POC test, and, anecdotally, women preferred this approach to self-collected and clinician-collected samples. We found favorable participation in community-based screening. Additionally, in rural, low-resource settings, DBS is easier to transport than slides, urine, or liquid-based cervico-vaginal samples, requires no refrigeration, and can potentially provide a "woman's physical" in a self-collected sample.

Our proposed study would be the first to rigorously examine the utility and feasibility of using menstrual blood collected by women for detection of HR-HPV DNA and HPV E6/E7 mRNA, using the Q-pad. We hypothesize that using self-collected menstrual blood from the Q-pad may be a more convenient, comfortable and cost-effective method for HR-HPV and E6/E7 screening, compared to currently implemented approaches, with high correlation to conventional HR-HPV testing. Our approach to screening may also allow for improved access to screening for cancer and precancer in the areas with the highest burden of disease, including unscreened women in the US and worldwide.

3.0 STUDY ENROLLMENT AND PARTICIPANT ELIGIBLITY

The participant population to be included in the study is menstruating women over the age of 18. There will be two groups: 1) menstruating women over the age of 18 who have a previous history of HR-HPV in the last 18 months and 2) menstruating women over the age of 18 who do not have a previous history of HR-HPV. We plan to recruit patients from Stanford research IT Starr cohort tool to identify patients who have had a positive

HR-HPV test in the past 18-months. In addition, we also plan to recruit women without previous history of HR-HPV, who present in clinic for a cervical cancer screening test as part of their standard of care well woman visit.

3.1 Study Enrollment

For positive HR-HPV participants:
We plan to recruit patients from Stanford research IT Starr cohort tool to identify patients who have had a positive HR-HPV test in the last 18-months. We will then contact their provider to get permission to include them in the study before their next in-person follow up. This follow-up visit can be any time after positive HR-HPV result up to 18-months. This follow-up visit can be for a repeat cervical cancer screening pap test, colposcopy, or loop electrosurgical excision procedure. Patients identified who are HR-HPV positive will be contacted by their provider to broach the possibility of research participation to the patient. If this patient expresses interest in the project, the provider will ask if the research staff can then contact the patient. If the patient agrees, then the research staff will contact the patient. In addition, patient's with known positive HR-HPV results who present to the clinic for colposcopy or loop electrosurgical excision procedure will be approached by their provider during that clinic visit to see if the patient would be willing to participate in the project. If the patient agrees, then research staff will present the project in detail to the patient and obtain consent. Pre-procedure meetings (either in person or over the phone) will be performed with the subjects individually, where the process will be discussed and instructions on how to use the Q-pads and the vaginal swabs will be explained to ensure women fully understand the process. We will also hand out (or mail) instructions with pictures on paper to minimize any risk associated with using the devices. We do not anticipate any screen failures. Eligibility is based on standard of care testing.

For participants with no prior HR-HPV history:
We plan to recruit patients from Stanford Gynecology clinic. We will review the clinic schedule for patients presenting to the clinic for their routine well woman visit with cervical cancer screening. Their provider will approach the patient during that clinic visit to assess if the patient would be willing to participate in the project. If the patient agrees, then research staff will present the project in detail to the patient and obtain consent. At the time of the routine cervical cancer screening, additional study swabs will be collected. Prior to discharge, research staff will show the subject how to use the Q-pads and the vaginal swabs to ensure subjects fully understand the process. We do not anticipate any screen failures.

3.2 Participant Criteria

The inclusion criteria are women older than 18 years, who regularly menstruate and have tested HR-HPV positive in the last 18-months. Further, we intend to include women older than 18 years who regularly menstruate and have not been tested HR-HPV positive in the last 18-months. The exclusion criteria are women younger than 18 years old or who are post-menopausal or not menstruating regularly.

3.3 Enrollment

The target number of subjects at our gynecology clinic is 250 patients with or without previous HR-HPV diagnoses. We expect to complete this study within 12 months.

4.0 MATERIAL AND METHODS

Pre-procedure

Pre-procedure meetings will be performed with the subjects individually where the process will be discussed and two designated menstrual pads (pad 1 and pad 2) and two pouches (pouch 1 and pouch 2) will be dispensed. We will instruct the subjects on how to use the pads and go through the consent form with them and answer any questions they might have.

Self-Sample collection

Participants will use the pads as close to their scheduled, standard of care appointment or follow-up appointment —either during the menstrual cycle before or after the appointment. When the woman has her period, she uses the pads following instructions given to her orally and in written format during the pre-procedure meetings.

The Q-pad is used like any other menstrual pad. After usage you pull a small slip which removes a small paper-based collection strip. She will dispose the pad and put the collection strip into the provided envelope.

The strip will be put in a pre-stamped envelope and into a mailbox and shipped to a CLIA waived and CAP certified laboratory for analysis. Results will be emailed back to the investigators in a coded fashion. DNA will not be extracted from the samples and the Stanford Tissue Bank will not be used for the banking of specimens. Samples will be disposed of after completion of the current study.

Provider sample collection

Participants will attend a scheduled, standard of care visit with their provider either for screening of HR-HPV or for follow-up within 18-months of the positive HR-HPV test for a repeat pap smear, colposcopy, or loop electrosurgical excision procedure. The provider will be asked to collect an additional sample for the study, which will be shipped to a laboratory for HR-HPV analysis. Additionally, at this time the participant will be asked to obtain two “self-swabs”. While in clinic, she will be administered swab device kits with verbal and written instructions.

We are using a commercially available menstrual pad. The menstrual pad is made of organic cotton and is today commercially available in convenient stores such as CVS and Walgreens. The only difference to a normal product is a small strip that is embedded beneath the first cotton layer. The strip consists of medical grade materials manufactured according to the ISO 13495 standard for medical devices. The core material in the strip is a proprietary paper-based sample receiving material from GE, namely a Whatman 903 collection paper. This is a commercially available product.

Follow-up
After we have received the sample collected by the participant and the provider, the subject will receive compensation.

5.0 STATISTICAL CONSIDERATIONS

5.1 Outcome Measurements

Primary outcome is to compare the accuracy and percent analytic agreement of clinician and self-collected cervico-vaginal brush samples with Q-pad-collected menstrual blood for HR-HPV detection in women with and without previous positive HR-HPV results. The secondary outcome is to assess the ability to detect oncoproteins in Q-pad collected menstrual blood samples from women with ASCUS+ to determine the utility in cervical cancer/precancer detection and to test the utility of a low and high threshold HR-HPV reflex scheme to similarly increase the specificity of HR-HPV testing derived from menstrual blood. Neither outcome measures relate to safety.

5.2 Analysis plan

We will record all data on an electronic tablet using REDCap electric data capture tools hosted at the Stanford Center for Clinical Informatics. 25 IBM SPSS Statistics, version 25.0 (IBM Corp., Armonk, N.Y., USA) will be used for data analysis. We will assess sociodemographic and clinical characteristics using descriptive statistics; if comparisons are needed, chi square tests or Mann-Whitney U tests will be used, where appropriate. We will assess the percent agreement of the binary outcome of HPV positive or negative on the menstrual blood sample analysis compared to the clinician swab and the self-swabs using Cohen's kappa.

5.3 Sample size

We chose a convenience sample of 250 women based on the following: Stanford Gynecology clinic has approximately 10,000 unique visits per year. A "worst case" HPV prevalence rate of 5% (similar to rural Thailand[14]), yields approximately 500 positives for HR-HPV per year and recruitment experiences from similar studies indicates a recruitment rate of 50% of the positives for something like this is feasible. In fact, the local HPV prevalence rate in our catchment area is likely much higher than 5%. Also 250 women would allow for adequate precision of 95% relative to agreement of the menstrual specimen with the lab-generated HPV result (alpha =.001, beta=.10)[15].

6.0 DATA MANAGEMENT CONSIDERATIONS

6.1 Data management

REDCap will be used for data collection and measures taken to protect identifying

information when exporting data. A unique study ID will be used in all statistical analyses to protect confidentiality. All electronic devices used in data collection will be both encrypted and password protected through Stanford IRT.

Adverse Events, protocol deviations, aggregate data, and individual data will all be reviewed by the research team at monthly intervals or as necessary. The Principal Investigator, Dr. Paul Blumenthal, with the help of the Family Planning Division Research Team will be responsible for Data and Safety Monitoring. Assessments of data and events captured by the monitoring will be performed on a continual bases, simultaneously with the reporting data from analysis sources (i.e. physician office visits, follow up phone calls). If there is an unanticipated problem (UP; unexpected, related to research, AND harmful), it will be promptly reported to the IRB. Dr. Paul Blumenthal, as the Data Safety Monitoring person, will disseminate the outcome of all reviews to the IRB.

6.3 Confidentiality

Participants in the study will be seen in private rooms for enrollment. No PHI will be left on participant's voicemail and/or email. Prior to enrollment, participants will receive HIPAA training and will work to protect the privacy interests of participants. A PHI secure website application will be used for data collection and measures taken to protect identifying information when exporting data. A unique study ID will be used in all statistical analysis to protect confidentiality. All study personnel with direct access to subjects' medical records will take reasonable precautions within the constraints of applicable regulatory requirements to maintain the confidentiality of the subjects' identity.

6.4 Protocol Review and Amendments

The protocol, the proposed informed consent and all forms of participant information related to the study (e.g. advertisements used to recruit participants) will be reviewed and approved by the Stanford IRB and Stanford Cancer Center Scientific Review Committee (SRC). Any changes made to the protocol will be submitted as a modification and will be approved by the IRB prior to implementation. The Protocol Director will disseminate the protocol amendment information to all participating investigators.

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APPENDIX A**: Participant Eligibility Checklist**

Protocol Title:	**Screening for Cervical Intraepithelial Neoplasia Using Self-Collected Menstrual Blood**
Protocol Number:	**47250**
Principal Investigator:	**Dr. Paul Blumenthal**

II. Subject Information:

Subject Name/ID:
Gender: ☐ Male ☒ Female

III. Study Information:

☒ SRC Approved ☒ IRB Approved ☒ Contract Signed

IV. Inclusion/Exclusion Criteria

Inclusion Criteria (From IRB approved protocol)	**Yes**	**No**	Supporting Documentation*
1. 18 years or older	☐	☐	
2. Menstruate regularly	☐	☐	
3. Comfortable using menstrual pads	☐	☐	
4. Comfortable using vaginal swabs	☐	☐	
5. Comfortable committing to using condoms from time of self-collection and provider collected sample	☐	☐	
Exclusion Criteria (From IRB approved protocol)			
1. Women younger than 18 years old	☐	☐	
2. Women who are post-menopausal or not menstruating regularly	☐	☐	

*All subject files must include supporting documentation to confirm subject eligibility. The method of confirmation can include, but is not limited to, laboratory test results, radiology test results, subject self-report, and medical record review.

IV. Statement of Eligibility

By signing this form of this trial, I verify that this subject is [☐ **eligible** / ☐ **ineligible**] for participation in the study. This study is approved by the Stanford Cancer Institute Scientific Review Committee, the Stanford IRB, and has finalized financial and contractual agreements as required by Stanford School of Medicine's Research Management Group.

Treating Physician Signature:	Date:
Printed Name:	

Secondary Reviewer Signature:	Date:
Printed Name:	

Study Coordinator Signature:	Date:
Printed Name:	

APPENDIX B: **Protocol Summary of Changes**

Any administrative changes to the protocol including spelling corrections, minor clarifications, renumbering, and reformatting are not summarized in the following table.

Section	**Change to Protocol**	**Rationale**
Title Page	Date and version # changed	Changed due to this revision
Specific Study Aims	Added samples from women with no previous history of HR-HPV to specific aim #1	The study team assessed the funding timeline and the current sample (n=70) and the goal of n=250 and identified the need to expand the inclusion criteria. By including those without a history of HPV, we are able to better assess specificity and sensitivity. One challenge our team has faced is that far less women menstruate regularly than anticipated—this is largely due to contraceptive use.
Study Enrollment and Participant Eligibility	Created two groups for study enrollment to include women with no previous history of HR-HPV	To collect as much information as possible given funding timeline restraints. See above.
Study Enrollment and Participant Eligibility	Changed age restrictions to include for women over 45 years of age.	To allow for menstruating women over the age of 45 to participate. Upon review of our age eligibility, we saw no reason to exclude those over 45 years of age who menstruate. Screening still occurs in this population.
Material and Methods	Changed methods to include approaching women presenting to clinic for repeat cervical cancer screening with or without a history of HR-HPV.	To expand enrollment.
Statistical Considerations	These changes will not impact the statistical methods.	To accommodate expanded enrollment.

Data Management Considerations	Added section 6.4	To provide clarity on amendment approval.