Lavage of the Uterine Cavity for Diagnosis of Ovarian Cancer

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STUDY PROTOCOL

LAVAGE OF THE UTERINE CAVITY FOR DIAGNOSIS OF OVARIAN CARCINOMAS

PRINCIPAL INVESTIGATOR: Barbara Norquist, MD
TITLE: Associate Professor
INSTITUTION: University of Washington
ADDRESS: 1959 NE PACIFIC ST, BOX 356460
Seattle, WA 98195
Phone: 206-616-1620
Email: bnorquis@uw.edu

CO-INVESTIGATOR: Rosa Ana Risques, PhD
Title: Assistant Professor
INSTITUTION: University of Washington
ADDRESS: 1959 NE PACIFIC ST, BOX 356460
Seattle, WA 98195
Email: rrisques@uw.edu

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SUMMARY OF PROPOSED RESEARCH

The goal of this project is to develop a minimally invasive test to detect ovarian cancer, by searching for mutations from the tumor in samples obtained from the cervix (Pap smears), and from the uterus (uterine lavage) in women with ovarian cancer and women with a genetic predisposition to malignant neoplasm of the ovary. We plan a pilot study of 60 women with ovarian cancer and with genetic predispositions to malignant neoplasm of the ovary. Pap smear and uterine lavage samples will be collected while the woman is under anesthesia for planned debulking surgery or prophylactic surgery. A novel, highly sensitive and accurate technique, Crispr-Duplex sequencing, will be used to detect tumor associated mutations in *TP53* (the most commonly mutated gene in ovarian cancer) within these samples. These results will be compared to sequencing results in the tumor itself for comparison, and Pap and uterine lavage will be compared to each other to determine the optimal test.

A. BACKGROUND AND SIGNIFICANCE

In 2017**,** more than 22,000 women will be diagnosed with ovarian cancer and more than 14,000 women will die from the disease, making it the leading cause of death from gynecologic cancer[1]. Ovarian, peritoneal, and fallopian tube carcinoma can clinically be considered one disease, referred to collectively as OC. The majority of patients present with advanced stage III or IV disease, with 5-year survival rates of 34% and 18%, respectively[2]. In contrast, patients diagnosed with stage I disease have 90% 5-year survival[2], demonstrating the critical importance of early detection. Efforts at screening for early stage OC using ultrasound and serum

biomarkers such as CA-125 have been unsuccessful at reducing mortality from this disease[3, 4]. Women with inherited mutations in *BRCA1* and *BRCA2* are at increased risk of developing OC, with lifetime risks estimated near 50% for *BRCA1* and 20% for *BRCA2*[5, 6]. Therefore, for high-risk women, national guidelines recommend removal of the ovaries and fallopian tubes after completion of childbearing[7]. Surgical prevention dramatically reduces the incidence of OC in *BRCA1* and *BRCA2* mutation carriers, and improves all-cause mortality[8, 9]. Risks of surgery, premature menopause, and loss of childbearing potential are balanced against these benefits. Women at high risk need more sensitive and specific screening options when they need to delay surgery. Mathematical models have estimated that women with *BRCA1* and *BRCA2* mutations who go on to develop OC spend at least 4 years with pre-invasive or early stage (I and II) lesions[10], providing a window in which an adequately sensitive screening method could lead to early detection. As these lesions are often microscopic or <1cm, they are not detected clinically by standard imaging techniques, and many early lesions will have a normal CA-125.

The limitations of CA-125 and ultrasound have led investigators to consider molecular approaches to early detection. The first demonstration that OC could be detected using minimally invasive sample collection came from Kinde et al, sequencing DNA from Pap smears[11]. Using an error-corrected next generation sequencing (NGS) method called Safe-SeqS, they were able to detect mutations in tumor DNA in 41% of Pap smears from women with OC. To improve the yield of tumor DNA, Speiser and Zeillinger developed a uterine lavage technique that allows for collection of samples from closer proximity to the OC. As the ovaries, fallopian tubes, and uterine cavity are in communication with each other, they hypothesized that uterine lavage could reliably capture OC DNA. They sequenced DNA from uterine lavage samples using NGS for a panel of potentially mutant genes, and were able to identify OC related mutations in 18/30 (60%) cases, with most being in the gene *TP53*[12]. Additional tumor mutations were identified in an additional 6 uterine lavage samples that were tested with digital droplet PCR (ddPCR), once the tumor specific mutation was determined by sequencing the OC itself. While this demonstrates an important proof of principle that the mutation can be detected, it is not helpful as a screening test as it would require a biopsy of the OC first. Given these studies' limitations in sensitivity, other sequencing techniques are needed in order to make this type of testing feasible.

Next generation sequencing techniques commonly introduce low-level errors that can appear to be mutations, masking true tumor-associated mutations that are present at very low frequencies, as would be expected for early stage cancers. In order to not miss these true mutations, substantial improvements to these methods are needed. Duplex Sequencing (DS), developed in the Loeb lab at the University of Washington[13], overcomes this problem by reducing the error rate to <1 in 10 million. This accuracy is accomplished by independently sequencing the two strands of each DNA molecule, as true mutations will be present at the same position in both strands, and artifacts or errors are present in only one of the two strands. Dr. Risques and colleagues have utilized DS to accurately detect mutations as infrequent as $4x10^{-4}$ in tumor DNA taken from peritoneal fluid samples in women with OC[14]. They then began collaborating with the investigators that developed the uterine lavage technique described above, and sequenced *TP53* in uterine lavage samples from OC patients with DS, identifying mutations in 8/10 (80%) samples (see preliminary data). *TP53* is mutated in >96% of high-grade serous OCs[15], in addition to most serous tubal intraepithelial carcinomas (STICs)[16], making *TP53* an optimal target for OC early detection. The main difficulty with DS is that efficiency is

low (~1%), requiring at least 1µg of DNA to achieve adequate sequencing coverage. Utilizing Crispr- Cas9 genome fragmentation, rather than random sonication, increases efficiency and requires much less input DNA.

Preliminary data:
In collaboration between the University of Washington and the Medical University of Vienna, DS was applied to 10 uterine lavage samples from women with OC, and 11 controls. Tumor associated *TP53* mutations were detected with DS in 8/10 (80%) women with OC, and not in controls, manuscript in preparation by Risques et al. Interestingly, biological background mutations in *TP53* were found in all tested subjects, including the healthy controls, a finding that was also present in the previous study of DS using peritoneal fluid from women with and without OC[14]. Tumor related *TP53* mutations tended to have higher allele frequencies, and in this study a cutoff of 0.01 was effective at differentiating between these two findings. Moreover, this threshold allowed for discrimination between women with OC from controls with 70% sensitivity and 100% specificity, a diagnostic performance superior to any other OC test currently available. Preliminary data suggests that filtration of the uterine lavage samples to remove clusters of endometrial cells results in an increased allele frequency of tumor associated mutations, and ongoing efforts are optimizing this protocol. This study provides evidence that uterine lavage combined with DS increases the sensitivity of tumor *TP53* mutation detection over other sequencing methods.

B. General Description of the Study
This study aims to collect specimens from 50 women with high-grade serous OC and 10 women who are undergoing prophylactic surgery for genetic predisposition to malignant neoplasm of the ovary, while they are under anesthesia for planned surgery at a single site, the University of Washington. There will be a total of at least 60 participants in the study.

Dr. Norquist will coordinate the collection of samples including a Pap smear, uterine lavage, and tumor at the time of planned primary surgery. DNA will be extracted from all samples. Dr. Risques will coordinate the *TP53* sequencing utilizing the Crispr-DS method on the Pap smear and uterine lavage extracted DNA. Primary tumors will be sequenced in order to confirm that the mutations identified in the Pap and/or uterine lavage are present in the tumor. We will compare the sensitivity for OC detection of Pap smear compared to uterine lavage, in order to optimize our assay.

Results will not be released to either patients or clinicians, or used for any treatment decisions.

C. Study Objectives

Objectives:
1) To collect samples from 50 women with high-grade serous OC and 10 women who are undergoing prophylactic surgery for genetic predisposition to malignant neoplasm of the ovary, including Pap smears, uterine lavage, and a tumor sample.
2) To test the DNA from Pap smears and uterine lavage samples for tumor-derived *TP53* mutations, using the new technology of Crispr-Duplex Sequencing.
3) Determine the sensitivity and specificity of Pap smear or uterine lavage in detection of tumor-derived *TP53* mutations.

D. Study Design

Study population and recruitment:

Patients with suspected OC and women who are undergoing prophylactic surgery for genetic predisposition to malignant neoplasm of the ovary will be identified and consented pre-operatively for participation in the study. This process is already in place at the University of Washington as nearly all patients with OC or those undergoing prophylactic surgery for genetic predisposition to malignant neoplasm of the ovary are consented pre-operatively for our Gynecologic Oncology Tissue Bank.

Eligibility:

Inclusion criteria:

- Women over the age of 18 with suspected ovarian cancer or genetic predisposition to malignant neoplasm of the ovary
- Planned surgery
- Have a uterus and no history of tubal occlusion

Exclusion criteria:

- Unable to speak English
- Unable to provide informed consent
- Prior hysterectomy

Sample collection:

After induction of anesthesia, samples will be collected, starting with a standard liquid-based Pap smear. Uterine lavage is then carried out. First the cervix is cleansed with a betadine solution as is standard prior to surgery involving the uterus. The three-way endometrial catheter is then inserted into the cervical canal and 10ml of sterile saline are used to irrigate the uterine cavity, as previously described[12]. The three-way uterine lavage catheter developed by Oncolab Diagnostics has received FDA designation as a nonsignificant risk (NSR) device. It is commercially available, and is currently being utilized in 3 ongoing clinical trials (NCT02039388, NCT02062697, and NCT02518256). Finally, samples of the OC itself are collected for those with suspected OC during the abdominal surgery for comparison. Uterine lavage samples will be filtered to remove clusters of endometrial cells to decrease the amount of non-tumor DNA.

Molecular analysis:

DNA will be extracted from each of the three samples collected per case (Pap, uterine lavage, and tumor). Pap smear and uterine lavage samples will be sequenced for *TP53* mutations using the Crispr-Duplex Sequencing method. Patients will be excluded from the study if the diagnosis of high-grade serous OC is not confirmed by the final pathology results.

Data collection:

Baseline demographic and clinical variables will be collected on each patient per procedures already in place with our gynecologic oncology Tissue Bank.

Data privacy and Safety Monitoring:

There will be limited access to authorized users who will use password protection and encrypted storage devices. Data will be stored in a locked cabinet on an encrypted flash drive. Access to electronic records are password protected. Team members will complete confidentiality and security training. No identifiers will be used in any study reports, publications or presentations.

Adverse Events:
Adverse events are unlikely with this study. Enrolled subjects are women who are already planned to undergo surgery for their suspected ovarian cancer, and they will be under anesthesia during collection. Collection is designed to occur in parallel with other portions of the surgery, so we do not anticipate it will prolong surgical time. Uterine perforation or bleeding, or a pelvic infection could occur, although it is unlikely based on the known safety of procedures such as endometrial biopsy. If any such adverse events occur, they will be reported.

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