# Screening for early familial ovarian cancer with proteomic pattern analysis and transvaginal ultrasonography

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## A. Study Purpose and Rationale

Ovarian cancer continues to be the leading cause of death form gynecologic malignancy, and the fifth most common malignancy among women, in the United States. To place this in perspective, more American women die from ovarian cancer each year than from homicide and suicide combined.[1] Annually, approximately 23,400 cases are diagnosed, and 13,900 deaths will occur. Furthermore, in contrast to advances being made against other malignancies, mortality rates for ovarian cancer have remained quite stagnant over the last two decades. [2]

It has long been recognized that screening for ovarian cancer, were it to be effective, could potentially drastically reduce the impact of the malignancy. A relatively rapidly progressive malignancy, one without a recognized pre-malignant state, stage at diagnosis is the strongest predictor for mortality. Indeed, stage I ovarian cancer, as defined by the 1988 Revised International Federation of Gynecologic Oncologists |FIGO|,[3] in which the malignancy is limited to the ovaries without extension, carries an excellent prognosis - an estimated 5-year survival rate in excess of 90% - while extension lowers this dramatically. Extraovarian but intrapelvic extension confers an estimated 5-year survival of 60%, metastasis to regional nodes is associated with a 5-year survival of 20%, and by the time the cancer has metastasized, 5-year survival has droppe~ to below 10%.[4] Despite the good prognosis seen in early disease, the cumulative 5-year survival rate for American women with ovarian cancer is only 30%, bespeaking the fact that the disease is most often identified only after significant progression has brought it to medical attention.

That a high-risk population of women exists, one that may particularly benefit from cancer screening, has both driven screening research and served as an important tool in assessing screening effectiveness. Given the higher prevalence of ovarian cancer among this high-risk population, defined as women who have either a family history positive for two or more first- or second-degree relatives with ovarian cancer or an identified ovarian cancer syndrome by genetic analysis (including the three most commonly identified mutations, BRCA 1, BRCA2, Lynch syndrome 11 a.k.a. HNPCC with non-colorectal malignancy, as well as at least five rarer syndromes, including gonadal dysgenesis XY, Peutz-Jeghers syndrome, Ollier's disease, the basal cell nevus syndrome, and familial ovarian fibromatosis),[5] the need for effective screening is greater and the required specificity to yield an acceptable positive predictive value is lower.

Nonetheless, clinical research to date has not been able to produce a screening regimen that is appropriate for women at average risk for ovarian cancer. The bulk of clinical investigation to date has focused on individual serum markers, such as CA-125, or imaging procedures, such as transvaginal ultrasonography. No single screening test has yet been shown to have an acceptable sensitivity, specificity, or positive predictive value for screening average risk women: CA- 125 $\geq$ 30 μg/ml has only 50% sensitivity for stage I ovarian cancer (although this rises to 90% for stage II), is notoriously non-specific among premenopausal women, and even among postmenopausal women has a specificity of approximately 98% -leading to positive predictive values of less than 3% for average-risk women and under 10% even among the high-risk population.[6] Sonography has been evaluated as a screening modality, but has not fared any better; reported sensitivities of 80-100% have been seen, with specificity ranging from 94-97%; however, again, positive predictive value has been unacceptably low (3-10%).[7,8] One trial assessing a combination of CA- 125 and sonography in postmenopausal women has been promising, and one randomized prospective trial of this approach is being performed - preliminary data, however, have failed to show a mortality benefit.' Despite these shortcomings, however, and despite the

formal USPSTF recommendation not to screen for ovarian cancer, the community standard-of-care is to screen women with known hereditary ovarian cancer syndromes with transvaginal ultrasound plus or minus CA- 125 - in keeping with one consensus conference's recommendations.[10]

It is against this backdrop that Petricoin and Liotta reported a small pilot investigation ,into the application of proteomic technology to screening for ovarian cancer. In their study, they developed a bioinformatics tool that they trained upon 100 patients, 50 identified to have known ovarian cancer and 50 known not to have ovarian cancer, identifying a spectral pattern in N-dimensional space that differentiated between the low-molecular weight proteins in the sera from patients with cancer and those in the sera of cancer-free patients. They then prospectively applied this discriminating spectrum to a masked sample of 116 patients, 50 women with ovarian cancer and 66 women with non-malignant gynecologic disease or no gynecologic disease. They found that, applied to this masked set, their proteomic spectrum (PS) correctly identified 50/50 cancers - including 18/18 stage I - and incorrectly labeled 3 of the 66 "normals" as positive, providing a specificity in this sampling of 95% and a positive predictive value (PPV) of 94%. The novel test compared quite favorable to CA-125 performed concomitantly, which achieved in the same masked patients a PPV of 35%.[11] Since the conclusion of this pilot investigation, the authors have continued to refine the instrument as they enter more known samples into the training database, and they believe that these further modifications will only increase the tests specificity without jeopardizing sensitivity. [12] Thus, at this time the technique of applying proteomic analysis to low molecular weight serum proteins seems primed for prospective investigation in high-risk patients.

**B. Study Design and Statistical Analysis**

This proposed study would be a prospective cohort study assessing the sensitivity, specificity, and positive predictive value of the PS in screening for ovarian cancer. The working hypothesis is that PS analysis will have a substantially greater PPV in the high-risk cohort than will ultrasound (or other previously reported techniques). Specifically, given a 3year incidence of ovarian cancer of 1.8% among these high-risk patients, the anticipated PPV of PS will be 64 percent, as opposed to 23 percent with ultrasound.

The planned methodology will be to enroll 1,446 high-risk patients over an anticipated 3-year period, at Columbia-Presbyterian Medical Center, Fox Chase Cancer Center, and the Huntsman Cancer Institute. Enrollees will be screened from new entries into the NIH Cancer Family Registry located at each of these three cites. Inclusion criteria will be female gender, age > 35, no prior ovarian malignancy, no prior oophorectomy, and either 1) a pedigree consistent with a familial ovarian cancer syndrome (two first- or second-degree family members with documented ovarian malignancy) or 2) a documented genetic screen positive for one of the above-mentioned familial ovarian cancer syndromes. Each prospective study enrollee will be presented the opportunity to be screened for this trial at time of enrollment in the CFR; those willing to undergo screening will be contacted by the study nurse specialist, who will perform a telephone pedigree screen and request that corroborating medical records be presented, although an absence of complete documentation will not preclude enrollment. The principle of informed consent will be strictly adhered to, informing the patient of study design, risks, benefits, and planned follow-up.

Patients who agree to enroll in the trial will have a time-zero PS assessed, concomitantly with the standard of care screening examination, transvaginal ultrasound. The results of the proteomic spectrum will be logged and7 maintained by an independent member of the study, and all data will be logged under confidential unique identifiers. Clinicians will be blinded to the results of the PS, but will have access to the results of the screening ultrasound (as they would under standard of care). Per protocol, all transvaginal ultrasounds will be performed with the Siemens Elegra 4201 with a 5 MHz transducer or comparable 4th generation ultrasound. All ultrasound real-time tapes will be independently reviewed by two radiologists at NYP, with a third radiologist adjudicating discrepancies. Abnormal findings will consist of either of the following criteria:

1) Ovarian volume greater than 8.8 ml
2) Ovarian echogenicity is not uniform

Any abnormal ultrasound will be repeated 3-6 weeks after the initial scan, during days 2-6 of the menstrual cycle in premenopausal women. If the repeat scan remains positive, the patient will be informed and referred to a gynecologic oncologist for diagnostic laparoscopy. Receiving diagnostic laparoscopy will be a patient's individual endpoint.

Patients with a negative ultrasound will continue to be seen in clinic annually for this study, at which time they will have repeat PS and ultrasound performed (again following the above parameters) at the end of the first, second, and third year of enrollment. Receiving a total of four negative annual screens will be the alternative individual endpoint. These repeat PS specimens will only be used for purposes of verification for cases in which time-zero PS is negative and ultrasounds in follow-up years 1-3 lead to biopsy confirmed diagnosis of ovarian cancer; they are not themselves the primary object of study.

All pathologic specimens will be reviewed at NYP Department of Pathology by two independent board-certified pathologists, with a third pathologist adjudicating any discrepancies.

**a. Category determination**

Research of ovarian cancer screening is limited in part by the absence of a noninvasive gold standard for the determination of the presence or absence of the malignancy at a given time. Diagnostic laparoscopy or laparotomy is the current gold standard, but as an invasive surgical procedure carries risks of morbidity (2%) and mortality (0.2%). 13 Thus, it is challenging to demonstrate the truth of a negative screen without subjecting a patient to unnecessary risk. This being said, given the relative speed at which ovarian cancer progresses - both clinically and radiographically - it has often been suggested that absence of clinical progression in the 18-36 months after a negative screen can effectively retrospectively validate the negative result. With this caveat in mind, and appreciating the limitations that this places upon the study results, we will define true positive (TP), true negative (TN), false positive (FP), and false negative (FN) as below:

TP: A PS screen at time zero discriminated as "positive for cancer" and a biopsy confirmed malignancy within 36 months.

FP: A PS screen at time zero discriminated as "positive for cancer" and no onfirmed malignancy within 36 months.

TN: A PS screen at time zero discriminated as "negative for cancer" and noconfirmed malignancy within 36 months

FN: A PS screen at time zero discriminated as "negative for cancer" and a biopsy onfirmed malignancy within 36 months.

The 36-onth threshold is in fact longer than is recommended by some for such purposes, and indeed much research has elected the 24 month cutoff instead. Within this study, the long cutoff can be expected to minimize the number of patients incorrectly classified as false-positive, as there is time for a malignancy correctly identified by PS to become radiographically or clinically apparent. However, such a cutoff could be expected to lead to patients incorrectly being classified as false negative, as a malignancy could arise *de novo* between the time-zero screen and the conclusion of the study.

Thus, a secondary analysis is planned to analyze the group of false negative patients to assess the PS from years I-n, where n is the year in which malignancy was diagnoses, to quantify what percentage of these patients were "PS converters," moving from being classified as negative for cancer to positive for cancer prior to the biopsy confirmed diagnosis. In such a patient, the initial negative PS - within a programme of annual screens - would be classified as a true negative.

A similar post-hoc analysis is planned to vaiidate the true positive patients. It is theoretically possible that these patients could have a positive time-zero PS but have been free of malignancy at that time, only developing a *new* malignancy afterwards. This secondary analysis will be to assess what percent of TP patients maintain their PS positivity from time zero to year of diagnosis. It is anticipated that this number will be 100%.

**b. Statistical analysis**

The number of patients which the study design calls for, 1,446, was determined to provide this study 80% power at the $\alpha$=0.05 level to reject a PPV of 23% (the anticipated PPV of sonography in this high-risk population) for a true value of 64%. The assumptions underlying this calculation are that the 23% PPV of sonography is well known, thus enabling the group ratio to be modified, and that the 3-year incidence of ovarian cancer in this high-risk population will be 1.8%. The $\chi^2$ test will be used in the comparison of PPV of PS to ultrasound. Secondary analyses will include assessing the specificity and sensitivity (within the limits of the study) of PS for ovarian cancer and a comparison of this specificity and sensitivity to that of ultrasound, again through a $\chi^2$ analysis.

## C. Study Procedure

The study procedure will consist of only two interventions, transvaginal ultrasound and PS blood draw.

**a. Transvaginal ultrasound**

This imaging technique involves the placement of a thin ultrasound transducer into the vaginal canal and against the cervix and vaginal walls. This approach to evaluation of the pelvis and adnexa allows for clearer definition of anatomic structures than does transabdominal sonography, even in the presence of a liquid-filled bladder. The procedure is well tolerated and carries no morbidity or mortality directly from the intervention. The patient also receives no exposure to radiation.

**b. Proteomic spectral analysis blood draw**

As the microchips used for proteomic analysis with SELDI-TOF mass spectroscopy require only 10 µl of blood, all that will be required is a simple "fingerstick" as is performed by diabetics to assess blood glucose. Sterile disposable lancets will be used after the lateral tip of the third finger has been cleaned with a disposable alcohol pad and allowed to dry. Fingerstick technique carries no significant or quantifiable morbidity or mortality.

## D. Study Drugs

No drugs will be specifically prescribed within the trial protocol.

## E. Medical Device

Protein Biology System 2 SELDI-TOF (surface enhanced laser desorption and ionization time-of-flight) mass spectrometer (Ciphergen Biosystems)

Proteome Quest beta version 1.0 (Correlogic Systems Inc)

Elegra 4201 ultrasound with 5 MHz vaginal transducer (Siemens Inc) or equivalent

## F. Study Questionnaires

None

**G. Study Subjects**

See above. Men are excluded from the study, for what is felt to be a strong rationale.

**H. Recruitment of Subjects**

See above.

**I. Confidentiality of Study Data**

See above.

**J. Potential Conflict of Interest**

None.

**K. Alternative Therapies**

See above.

**L. Compensation to Subjects**

Subjects will receive parking vouchers for their annual visit and any visits for follow-up ultrasonography in the event of a positive ultrasound scan.

**M. Direct Costs to Subjects**

None.

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