Early Detection of Ovarian Cancer: Background, Rationale, and Structure of the Yale Early Detection Program

PETER E. SCHWARTZ, M.D.,[a] JOSEPH T. CHAMBERS, M.D., Ph.D.,[a]
KENNETH J. TAYLOR, M.D.,[b] JOHN PELLERITO, M.D.,[b]
LYNWOOD HAMMERS, D.O.,[b] LAURENCE A. COLE, Ph.D.,[a]
TERESA L. YANG-FENG, Ph.D.,[c] PHYLLIS SMITH, M.S.W.,[d]
SUSAN T. MAYNE, Ph.D.,[e] AND ROBERT MAKUCH, Ph.D.[e]

[a]*Department of Obstetrics and Gynecology,* [b]*Department of Diagnostic Imaging,*
[c]*Department of Human Genetics,* [d]*Department of Social Work,* [e]*Department of
Epidemiology and Public Health, Yale University School of Medicine and Yale–New
Haven Hospital, New Haven, Connecticut*

Ovarian cancer has received national attention as a highly virulent disease. Its lack of early warning symptoms and the failure to develop highly sensitive screening tests have led some physicians to recommend prophylactic oophorectomies to women with relatives who have had ovarian cancer. Others have recommended routine screening of otherwise normal women for CA 125, a circulating tumor marker, and ultrasound examinations. Each of these techniques is associated with substantial false-positive rates that could lead to unnecessary surgery. A review of epidemiologic data suggests that familial ovarian cancer kindreds are rare, but women with first-degree relatives who have had ovarian cancer have a significant risk themselves for developing ovarian cancer. In addition, women with a great number of ovulatory cycles are at an increased risk for the disease. Circulating tumor markers are frequently elevated in women with advanced ovarian cancer, but their value in early detection of ovarian cancer has yet to be established. Advances in endovaginal ultrasound and color Doppler flow technology have significantly improved our ability to assess pelvic organs. This article presents the background, rationale, and structure of the Yale Early Detection Program for ovarian cancer, whose goals are to identify the best techniques for diagnosing ovarian cancer in an early stage, to determine the frequency with which such tests should be employed, to assess false-positive results, and to identify women who might benefit from prophylactic oophorectomies.

INTRODUCTION

Ovarian cancer is the fifth most common cancer in American women but the fourth leading cause of cancer death [1]. Among women with pelvic reproductive cancers, more deaths will occur from ovarian cancer than from cervical and uterine cancer combined. The American Cancer Society estimates that approximately 20,700

new cases of ovarian cancer will develop in 1991, and 10,500 women will die in 1991 from this disease [1]. The high mortality from ovarian cancer reflects a lack of early warning symptoms and a lack of an effective screening procedure. Thus, 70 percent of women with common epithelial ovarian cancers, which represent approximately 90 percent of all ovarian malignancies, will present with International Federation of Gynecologists and Obstetricians (F.I.G.O.) stage III or IV disease, as symptoms routinely develop only when the cancer has spread to the upper abdomen (stage III) or beyond (stage IV) [2]. The five-year disease-free survival for patients with stage III and IV cancer is approximately 20 percent. Dramatic improvements in treatment, including aggressive cytoreductive surgery and aggressive chemotherapy, have led to prolonged survival, but the overwhelming majority of patients with stage III or IV disease ultimately succumb to the cancer [2–6]. Patients with ovarian cancer diagnosed as F.I.G.O. stage I (limited to the ovaries) or II (limited to pelvic metastases) disease have, however, five-year survival rates of 90 and 70 percent, respectively [7,8]. Despite much publicity and campaigns to implement screening programs, there is no evidence at this time that screening the population at large is an effective approach for the early detection of ovarian cancer.

Although there has not been an increase in the incidence of ovarian cancer, there has been an explosion of information transmitted to the public about this disease. The recent deaths of Governor Ella T. Grasso of Connecticut and Gilda Radner have focused attention on ovarian cancer in American women. Recent attention in the mass media has focused on the virulence of ovarian cancer, a role for a tumor marker, CA 125, and ultrasound screening for its early detection. The implications of false-positive CA 125 assays and other biological marker tests, particularly in asymptomatic pre-menopausal women who request ovarian cancer screening, has caused confusion and conflict between patient and physician. The management of minimal (1–3 cm) ovarian cysts in post-menopausal women identified only by screening ultrasound examinations has led to unnecessary surgery. Of most concern, however, is a suggestion from the Gilda Radner Familial Ovarian Cancer Registry that women who have first-degree relatives with ovarian cancer have up to a 50 percent chance of developing the disease, compared to a 1.4 percent chance in women without such family histories [9]. This registry suggests routine prophylactic oophorectomy in such women. Data to support this recommendation, however, have not been provided. Nevertheless, women in Connecticut are already undergoing prophylactic oophorectomies based on this recommendation.

The fact that physician practice patterns are evolving in the absence of supporting clinical investigations creates an urgent need to establish a program to determine the most effective early detection procedures for ovarian cancer, the limitations of these procedures, and which women would benefit from prophylactic oophorectomies to avoid subsequently developing ovarian cancer. If one could identify a population of women at high risk for ovarian cancer, it would be possible to accomplish the aims of such a program.

BACKGROUND

Epidemiologic Factors

A group of women at high risk for developing ovarian cancer has now begun to be recognized. Lynch and colleagues have reported on familial ovarian cancer kindreds

[10,11]. These kindreds are infrequent in occurrence and often have other cancers associated with them, in particular breast cancer. The observed cases of ovarian cancer in these kindreds suggested an autosomal dominant pattern of inheritance. In a case-control study of 62 women between the ages of 45–74 admitted to seven hospitals in Connecticut, Hildreth et al. identified women with first-degree relatives (mother and sisters) experiencing ovarian cancer having an 18.2-fold (95 percent confidence limits, 4.8–69.0) elevation in risk for developing cancer of the ovary [12]. Cramer et al. estimated by empirical logic that the relative risk for ovarian cancer if a primary relative had an ovarian cancer was 11.32 (95 percent confidence limits, 8.87,-) [13]. In that study, no controls had a primary relative with ovarian cancer [13]. Schildkraut and Thompson have reviewed data from a population-based case-control study, the Cancer and Steroid Hormone Study, conducted by the Centers for Disease Control [14]. In this study, 493 women aged 20–54 who had epithelial ovarian cancer were compared to a group of 2,465 controls. Ovarian cancer in the first- and second-degree relatives of cases was significantly more common than in the relatives of controls, with the odds ratios for ovarian cancer in first- and second-degree relatives being 3.6 (95 percent confidence interval, 1.8, 7.1) and 2.9 (95 percent confidence interval, 1.6, 5.3), respectively, compared to women with no family history of ovarian cancer. Schildkraut and Thompson thought their data were more consistent with an autosomal dominant mode of inheritance than an autosomal recessive mode but suggested a polygenic mode of inheritance might account for the familial aggregation patterns observed [14].

Important characteristics of familial ovarian cancer are *(1)* a tendency for earlier age at onset, with the mean age being 47.7 years as compared to 59 years for the general population, and *(2)* a tendency to be overwhelmingly serous cancers (90–97 percent) that are histologically poorly differentiated [15,16].

Numerous studies in the last two decades have also identified the involvement of the process of ovulation as a risk for ovarian cancer [10–14,17–19]. The greater the number of ovulatory cycles, the greater the risk for ovarian cancer. Pregnancy is associated with anovulation. Therefore, the greater the number of pregnancies a woman has, the fewer ovulatory cycles she experiences and the lower her risk for developing ovarian cancer. Breast feeding is associated with prolonged postpartum amenorrhea. Thus, a woman who has breast-fed her children has fewer ovulatory cycles than one who has not and has a reduced risk for developing ovarian cancer [20]. Oral contraceptives function by preventing ovulation and serve a protective role with regard to developing ovarian cancer [17]. Finally, an early menarche and a late menopause increase the number of ovulatory cycles that a woman experiences, whereas a late menarche and an early menopause have the opposite effect and may be protective regarding ovarian cancer.

Other factors that have been associated with ovarian cancer risk include diets high in animal fat, ingestion of lactose, type A blood, childhood mumps, and the use of talc in feminine hygiene [21–23]. Dietary factors have been implicated in the etiology of numerous cancers; however, the data regarding diet and ovarian cancer risk are limited and often contradictory. A clearer understanding of dietary risk factors is needed, due to the lack of primary prevention strategies for ovarian cancer. Some dietary studies suggest that animal fat, but not vegetable fat, is positively associated with ovarian cancer risk [24,25]; however, others fail to find an association with total fat or type of fat [26]. Daily consumption of meat, a significant contributor to dietary

fat intake, has been associated with an increased risk for ovarian cancer in some studies [21,25–27]. Daily fish consumption has also been reported to increase risk in some studies [17,28], although others report an inverse association between ovarian cancer risk and fish intake [29]. Milk consumption is positively associated with risk in some studies [30], while others report a negative association with milk consumption [27,28]. Mettlin and Piver reported that, while total milk drinking was unassociated with risk, drinking whole-fat milk significantly increased risk, while drinking low-fat milk significantly reduced risk [31]. Other dietary factors reported to be protective against ovarian cancer include vegetables and/or beta-carotene [24,26,29], and cereals, whole grains, or pasta [29,30]. Finally, alcohol has been reported to decrease risk [32] or to be unassociated with risk of ovarian cancer [29,33].

Circulating Tumor Markers

Recent developments, particularly in monoclonal antibody technology, have resulted in the identification of circulating tumor markers that are frequently elevated in women with ovarian cancer. CA 125 has been the most intensively studied marker [34,35]. It is an antigenic determinant expressed by an approximately 200 kD glycoprotein that is detected by the monoclonal antibody OC 125. It is very highly expressed by derivatives of fetal coelomic epithelium, elevated in the sera of approximately 80 percent of women with ovarian cancer but is also elevated in approximately 60 percent of patients with pancreatic carcinoma and 20–25 percent of patients with other solid tumors [35]. Thus, it is not a specific tumor marker for ovarian cancer. It is also elevated in primary liver disease, particularly in association with ascites.

CA 125 levels reflect tumor volume and have been used in the evaluation of the success of cancer treatment. In ovarian cancer patients who appear to be in remission, serum CA 125 elevations may be the first indication of treatment failure. CA 125 has also been used in an attempt to distinguish benign pelvic masses from malignant ones. Elevations of CA 125 greater than 35 U/mL in women over 50 years of age in association with a pelvic mass are consistent with the diagnosis of ovarian cancer in 80 percent of women studied [36]. Elevation of CA 125 greater than 35 U/mL in women under 50 years of age in association with a pelvic mass is, however, associated with ovarian cancer in only 15 percent of women [36]. The false-positive rate in younger women is most often due to benign pelvic disorders such as endometriosis, uterine leiomyomata, benign ovarian cysts, and pelvic inflammatory disease. Thus screening women under age 50 exclusively with CA 125 will result in a high frequency of elevated marker values. An effective early detection program for ovarian cancer must consider incorporating a panel of circulating markers or alternative techniques such as pelvic ultrasound examinations.

Lipid-associated sialic acid in plasma (LSA) measures sialic acid present in the lipoprotein/glycoprotein fraction of plasma [37]. This fraction contains gangliosides and other cell membrane components. The assay methodology includes organic extraction of plasma and colorimetric determinations of lipid-bound sialic acid. Lipid-associated sialic acid in plasma was found to be elevated in a large group of women with ovarian cancer managed at Yale University School of Medicine [37]. Its elevation was similar to that of CA 125 in women with ovarian malignancies. Lipid-associated sialic acid in plasma is a nonspecific marker, however, and can be

associated with many malignancies. It is frequently elevated when an inflammatory process is present. Thus, it may play a role in the management of patients known to have cancer, but its interpretation in patients with pelvic masses is subject to limitations because of the nonspecific nature of this test.

Urinary gonadotropin fragment (UGF) or urinary gonadotropin peptide (UGP) is a small protein (molecular weight, 9,700) evincing sequence homology with segments of the human chorionic gonadotropin (hCG) beta subunit (molecular weight, 36,700) [38–40]. Like CA 125, it is produced primarily by ovarian cancers and, to a lesser extent, by endometrial, breast, and pancreatic malignancies [39,40]. Indeed, UGF tends to be found in the urine of women with ovarian cancer at about the same rate as CA 125 in studies performed at Yale University [38,39]. UGF may be elevated in patients whose tumors do not express CA 125 (detects >90 percent of those false-negative for CA 125) [40]. UGF may be useful in pre-operatively assessing young women with pelvic masses in whom CA 125 has already been established to be elevated. An elevated UGF assay, in association with a substantially elevated CA 125, strongly supports the diagnosis of an ovarian cancer, whereas, if the UGF is normal in the presence of a mildly elevated CA 125, a benign gynecologic process is more likely [40].

NB/70K is a glycoprotein extracted from human ovarian cancers. It was initially reported to be elevated in the sera of 60 percent of post-operative ovarian cancer patients [41,42]. Elevations of NB/70K are associated with advanced-stage disease and increasing residual tumor volumes. Unlike CA 125, NB/70K is not associated with any particular ovarian cancer histology or grade of differentiation. It was elevated in 45 percent (9/20) of stage I, well-differentiated, ovarian cancers [41]. NB/70K is also elevated in 33–41 percent of non-gynecologic cancers tested and in 21 percent of cervical and endometrial cancers [42]. We are currently incorporating NB/70K into a panel of tumor markers, including CA 125 and lipid-associated sialic acid in plasma, more effectively to establish pre-operatively the nature of pelvic masses in women.

Other markers that have been employed in the evaluation of adnexal masses include TAG 72, CA 15-3, CA 19-9, placental alkaline phosphatase (PLAP), and PLAP-like alkaline phosphatase [43]. TAG 72 is a glycoprotein detected by the monoclonal antibody B72.3, using an immunoradiometric assay [44]. The assay is not specific for ovarian cancer. TAG 72 does not tend to be elevated in the sera of women with falsely elevated CA 125 levels. It is not as sensitive as CA 125 in detecting ovarian malignancies but has been incorporated into a tumor marker panel for assessing women with pelvic masses to determine pre-operatively the nature of the mass [44]. The serum CA 15-3 immunoradiometric assay measures antigenic determinants on a glycoprotein of high molecular weight which are frequently associated with breast and ovarian malignancies [44]. These tumor-associated antigens are infrequently elevated in sera containing false-positive levels of CA 125. CA 15-3 has been incorporated into a panel of tumor markers for assessing the nature of pelvic masses [44]. CA 19-9, a carbohydrate determinant, has been assayed in serum from ovarian cancer patients and has a limited role in evaluating women with this disease [45,46]. It may be most useful in following patients with mucinous carcinomas of the ovary in whom CA 125 is frequently not elevated. PLAP and PLAP-like alkaline phosphatase are less likely to be elevated in the sera of women with

epithelial ovarian cancer than CA 125 but may be highly expressed in sera from women with endometrioid carcinomas of the ovary [43,44].

The lack of sensitivity and/or specificity of individual tumor markers for ovarian cancer has led to incorporating combinations of these markers (UGF and CA 125; CA 125, LSA, and NB/70K; CA 125, TAG 72, and CA 15-3, and others) in assessing patients with pelvic masses [40,43,46–48]. Multi-marker panels are likely to be more sensitive than single markers for distinguishing ovarian cancer from benign pelvic masses. The converse may, however, also hold true. If the markers are all normal, the patient may have a benign process. This possibility is extremely important information as women, who are at high risk for ovarian cancer and have adnexal masses, should be operated on by physicians prepared to perform aggressive debulking surgery and careful intra-abdominal staging procedures. Women with pelvic masses and a negative panel of circulating tumor markers are at low risk for gynecologic malignancies.

Ultrasound Screening for Ovarian Cancer

To date, the standard ultrasound examination of the ovary has been a transabdominal approach [49,50]. Campbell et al. recently reported on 5,479 self-referred asymptomatic pre- and post-menopausal women who were scheduled to undergo three annual transabdominal ultrasound screens evaluating ovarian size and morphology for abnormalities [49]. These women were not selected as being at high risk for ovarian cancer. Of the group, 326 women had positive results, but only five were identified as having ovarian cancer (four stage IA, one stage IB; two at screen 1, and two at screen 2; prevalence 0.09 percent) [49]. The odds that a positive transabdominal screen indicated the presence of an ovarian mass, a benign tumor, or any cancer or primary ovarian cancer were about 4 to 1, 2 to 1, 1 to 26, and 1 to 50, respectively. Recently developed high-resolution endovaginal ultrasound probes have become available; these devices adequately visualize both normal and abnormal ovaries [51,52]. These transducers yield much more information because of the proximity of the ovaries to the vagina, which allows the use of high-frequency resolution. Transvaginal sonography is easy to perform, as it does not require a full bladder, is well tolerated by women, and may accurately detect very small ovarian tumors [52]. The largest transvaginal ultrasound screening experience to date has been reported by van Nagell et al. [52]. One thousand asymptomatic women over age 40 have been evaluated, 75 of whom had an unspecified family history of ovarian cancer. Thirty-one patients in the entire series were found to have an abnormal vaginal sonogram, one of whom had an ovarian cancer.

Combined Tumor Marker and Ultrasound Screening

CA 125 assays have not been reportedly employed in the early detection of ovarian cancer in a high-risk population. Recently, CA 125 has been reported to be effective as part of an ovarian cancer detection program in 1,010 post-menopausal volunteers [53]. That program included vaginal examinations and transabdominal ultrasound examinations. One post-menopausal woman in the series was found to have a pelvic mass associated with an elevated CA 125 (32 U/mL) and an abnormal ultrasound examination. The mass proved to be a stage IA clear-cell carcinoma of the ovary. The specificity for the combination of CA 125 and vaginal examination with or without

ultrasound examination was 100 percent (95 percent confidence intervals, 99.6–100 percent) with a minimum follow-up of 12 months for all patients.

Prophylactic Oophorectomy in the Prevention of Ovarian Cancer

Prophylactic oophorectomy has been recommended for women in the rare breast-ovarian cancer family syndrome, where an autosomal dominant pattern of inheritance for ovarian cancer has been documented [11]. It must be stressed, however, that kindreds of breast and ovarian cancers occurring in families as described by Lynch et al. is a very unusual event [11]. Nevertheless, three of 28 such women who underwent prophylactic oophorectomies subsequently developed intra-abdominal carcinomatosis indistinguishable from ovarian cancer [45]. Anecdotal experience and personal observations suggest several reasons for this phenomenon's occurrence. One is an inadequate microscopic evaluation of the ovaries [54]. A second is incomplete resection of all ovarian tissue, as gynecologists do not routinely expose the retroperitoneal space and isolate the infundibulopelvic ligament vessels and ureter, as they try to avoid ureteric injury. In addition, most patients undergoing prophylactic oophorectomy have the procedure performed through small Pfannenstiel incisions or vaginal approaches, which are inadequate for the proper staging of an ovarian cancer.

THE YALE OVARIAN CANCER DETECTION PROGRAM

An ovarian cancer detection program concentrating on women at high risk for the disease is needed to identify the most effective procedures for the early detection of ovarian cancer, how often these tests should be employed, and how they should be interpreted. Screening recommendations developed for the high-risk group should be applicable for low-risk women seen in community practice settings. At present, circulating tumor markers and ultrasound examinations are being employed in a haphazard manner, at the discretion of clinicians and often at the urging of individual patients. The effect on health care costs for these services has yet to be assessed but undoubtedly is substantial. Information is also needed to develop criteria for recommending which women will benefit from prophylactic oophorectomy. At this time, it appears that prophylactic oophorectomy is appropriate in those rare kindreds where ovarian cancer is inherited in an autosomal dominant pattern [11]. The fraction of families with two or more first-degree relatives with epithelial ovarian cancer is somewhere between 0.7–5.8 percent of all families of ovarian cancer patients [55]. Evidence has yet to be provided demonstrating that any woman with a first-degree relative who has had an epithelial ovarian cancer has a 50 percent risk for developing ovarian cancer.

The purpose of the newly established Yale Ovarian Cancer Early Detection Program is to establish the value of a screening program for the early detection of this devastating disease. Women in Connecticut at high risk for ovarian cancer (those possessing first-degree relatives with a history of ovarian cancer), age 35 years or older, are invited to join the detection program. The objective is to try multiple methods to detect malignancy in its earliest stages (stage I and II), when it is still curable (five-year survival 90 percent and 70 percent, respectively) rather than at stages III and IV, its usual presenting stages, when survivability is poor (<20 percent). A panel of circulating tumor markers and diagnostic imaging techniques are studied in a serial fashion. Tests most useful in recognizing ovarian cancer will be identified and monitored for efficacy. Time intervals for utilizing these techniques

TABLE 1
Objectives

1. Evaluate the following studies to identify women who have early-stage ovarian cancer:
    a. A panel of contemporary putative tumor markers, including CA 125, LSA, NB/70K
    b. Investigational tumor markers, including colony stimulating factor-1 (CSF-1), serum beta-core
       fragment-carrier complex (BCF-COMPLEX)
    c. Urinary gonadotropin fragment (UGF)
    d. Endovaginal ultrasound studies
2. Monitor the value of serial circulating tumor markers and endovaginal ultrasound studies to establish:
    a. How often these fail by giving false-positive results in the absence of cancer
    b. The clinical strategies to assess the cause of these failures
    c. How often and which tests are best for the early detection of ovarian cancer
3. Identify individuals at high risk for ovarian cancer who may benefit from prophylactic oophorectomy
   by:
    a. Assessing their personal history, including medical, nutritional, and personal hygiene habits
    b. Their family history
    c. The results of circulating tumor marker values
    d. The results of endovaginal ultrasound studies
    e. Cytogenetic studies

will be established. In addition, epidemiologic data will be analyzed to determine which women might benefit from prophylactic oophorectomy (Table 1).

Participants are accrued through self-referral as well as through physician referral recruiting techniques. They become acquainted with our program through the mass media as well as via communication with physicians throughout the State of Connecticut by means of such mailings as the Yale Comprehensive Cancer Center *Caring*. This study will also be highlighted at the 1991 annual Ella T. Grasso Memorial Conference, a full-day symposium dedicated to new developments in the diagnosis and treatment of gynecologic malignancies, which is attended by many Connecticut gynecologists and medical oncologists.

Each year, the Division of Gynecologic Oncology at Yale University treats or consults on 50–70 new cases of ovarian cancer and is involved in the care of approximately 15–20 women, originally diagnosed and treated at other hospitals in Connecticut, who develop recurrent ovarian cancer. The families of women treated at Yale University are contacted, informed of the program, and invited to participate in it.

Participants call the registration secretaries for entry into the study and are screened through the initial telephone call to ascertain that they have at least one first-degree relative with ovarian cancer. Women eligible for participation are then sent an extensive medical and family history form to complete prior to being seen in the outpatient clinic facility. Once the completed medical and family history form has been received and reviewed by the clinical coordinator, the patient is invited to come to the Yale Gynecologic Oncology Center for further evaluation.

All participants are seen initially by a medical social worker, at which time an overall profile of the participant is obtained and further clarification and additions to the history obtained. The social worker in the Ovarian Cancer Detection Program, who provides the link between the medical members of the team and the participant, uses the participant's self-reported survey to enhance the psychosocial assessment and to provide consistent and broader meaningful histories. In our experience,

participants will frequently share more detailed information with a social worker who is not perceived as a deliverer of medical services. The social worker also communicates the details of the study to the participant in non-medical terms that are more easily understood.

The nursing staff then explain the nature of the laboratory tests to be obtained and answer any questions the participants may have regarding the Ovarian Cancer Detection Program. The participants then have blood drawn for circulating tumor markers and cytogenetic studies, and samples of white blood cells undergo DNA extraction for future molecular genetic studies. Plasma obtained at the initial visit is stored for future circulating tumor marker studies, as new markers become available. Urine is obtained for tumor markers. The subjects then undergo a physical examination by one of the physicians in the project, and any questions that they may have are answered. The physical examination is a routine complete physical examination, including a pelvic examination. Stool is checked for occult blood. If the participant has a normal examination and circulating tumor markers are normal (blood and urine), she is asked to return at three months for an endovaginal ultrasound examination and endovaginal color Doppler flow studies to evaluate the ovaries and the uterus. The subject returns at six months for a physical examination, including a pelvic examination and circulating tumor marker studies. The participant returns at nine months for a repeat endovaginal ultrasound and color Doppler flow study and, at the end of one year, for a repeat physical examination and study of circulating tumor markers (Fig. 1). The total costs for participation in the first year of the study are currently estimated to be $583.00 (Table 2).

Participants are informed by mail of normal laboratory results at the completion of each cycle of testing. They are called when abnormal laboratory results are evident. Referring physicians are also informed of the findings for their patients.

Blood and urine samples for the tumor markers selected for analysis in the Yale Ovarian Cancer Detection Program, CA 125, LSA, NB/70K, and UGF are obtained on entry to the study and at six months and 12 months later. Assays will be performed at Dianon Systems, Inc., a reference laboratory in Stratford, Connecticut. Plasma and urine are stored at Dianon Systems, Inc., to be utilized if new markers for ovarian cancer detection become available in the future. Blood and urine samples for research tumor marker assays are obtained at the same intervals. Blood samples obtained at the initial visit for the cytogenetic and molecular genetic studies are processed in the Department of Human Genetics at Yale University.

Participants with elevated tumor markers have the tests for the markers repeated in one month. If the markers are significantly elevated, i.e., CA 125 doubled, LSA increased >8 mg/dL, or NB/70K doubled, the participants promptly undergo endovaginal ultrasound evaluations. If the markers are not significantly elevated, participants continue with the established protocol.

The results of tumor marker testing will allow us to establish which markers are best for early detection of ovarian cancer. Moreover, it is our goal to determine the optimal timing for performance of such tests. Departures from stationary levels may require more frequent testing to evaluate whether a true departure from constancy is emerging, or whether the value arose simply as a consequence of variability, and there is a subsequent return to baseline constancy. Participants are more likely to have false-positive individual tests due to the nonspecific nature of the circulating tumor markers. By monitoring these tests in serial fashion, a data base will become
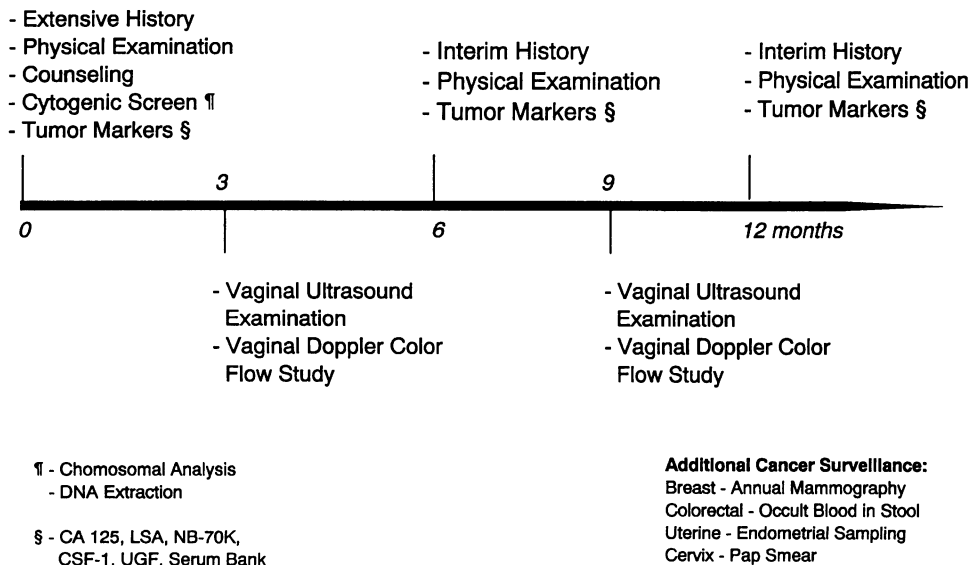
- Extensive History
- Physical Examination
- Counseling
- Cytogenic Screen ¶
- Tumor Markers §

- Interim History
- Physical Examination
- Tumor Markers §

- Interim History
- Physical Examination
- Tumor Markers §

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      |_____3_____|_____9_____|
      0                        |                6                |        12 months
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- Vaginal Ultrasound
  Examination
- Vaginal Doppler Color
  Flow Study

- Vaginal Ultrasound
  Examination
- Vaginal Doppler Color
  Flow Study

¶ - Chomosomal Analysis
  - DNA Extraction

§ - CA 125, LSA, NB-70K,
   CSF-1, UGF, Serum Bank

Additional Cancer Surveillance:
Breast - Annual Mammography
Colorectal - Occult Blood in Stool
Uterine - Endometrial Sampling
Cervix - Pap Smear

FIG. 1. Ovarian cancer screening program first-year flow diagram. **LSA,** lipid-associated sialic acid in plasma; **CSF-1,** colony stimulating factor 1; **UGF,** urinary gonadotropin fragment.

available to clinicians, one that will allow us to pursue alternate clinical strategies rather than promptly performing unnecessary oophorectomies in these women.

The diagnostic imaging technique to be employed primarily for screening in the Yale program is the endovaginal ultrasound [18,19]. Endovaginal ultrasound (EVUS) is used to examine the morphology and vascularity of the ovaries; the ovaries are located and carefully examined for size and morphology. The presence of any masses, cystic or solid, are noted and measured. The presence of any free peritoneal fluid is noted. After studying the morphology, color flow is utilized, and the ovaries again carefully examined for vascularity. Any arterial vascularity detected is quantitated by pulse Doppler. The Doppler signal is optimized to maximize peak systolic

TABLE 2
Early Detection Costs: First Year

1. Initial Visit	
History and physical examination	$105.00
Biomarker assays	$ 36.00
2. Three Months	
Ultrasound examination and clinical interpretation	$125.00
3. Six Months	
History and physical examination	$ 60.00
Biomarker assays	$ 36.00
4. Nine Months	
Ultrasound examination and clinical interpretation	$125.00
5. Twelve Months	
History and physical examination	$ 60.00
Biomarker assays	$ 36.00
Total Costs	$583.00

flow and signal amplitude. The signal is recorded and subjected to fast Fourier transform (FFT) to yield a time-velocity spectrum.

Signal analyses are then performed on line to yield the resistive index (RI), which is a semi-quantitative parameter of impedance. The spectra are also recorded on Super VHS for further analysis in the flow lab, yielding a LaPlace transform to characterize the signals better. Persistent low-impedance flow (RI < 0.5) has been found in our preliminary work and that reported from the group in London and Zagreb as being typical of neovascularity [56,57].

The timing of the examination is critical in pre-menopausal women. Taylor et al. first showed in 1985 that functional flow associated with the corpus luteum could be detected in the ovulating ovary by the pulse Doppler technique [58]. This low-impedance physiologic flow is similar to that seen in ovarian cancer, at least as far as resistive indices are concerned. Thus, it is important to avoid confusion by examining pre-menopausal women in the first eight days of their cycle, before luteal flow is manifest. If persistent functional flow is observed early in the cycle, repeat examination one month later usually demonstrates functional flow in the contralateral ovary. Timing is of less importance in the post-menopausal patient, who should not demonstrate functional flow. This flow may, however, be found in peri-menopausal women and also in patients following hysterectomy, and repeat scanning is important to differentiate between temporary physiologic flow and the pathologic neovascularity associated with ovarian cancer.

As far as morphologic criteria are concerned, a variety of different appearances are evaluated. Since the ovarian size varies with the patient's age, the size criteria for pre-menopausal women have to be more generous than those for post-menopausal patients. Particular attention is paid to the characteristics of any cyst, especially where mural thickening is apparent or where suspicion of malignant disease is heightened by the presence of abnormal vascularity and/or elevated serum CA 125 level. Differentiation between functional cysts and ovarian cancers should be possible by repeat scanning.

Participants with abnormal endovaginal ultrasound studies will have the findings reviewed in a multi-disciplinary bimonthly conference and correlated with physical examinations and circulating tumor marker determinations. Participants with findings compatible with ovarian cancer are recommended to undergo surgery. Those with equivocal findings by imaging have repeat imaging studies, including magnetic resonance imaging studies to define their abnormality further.

The results of the endovaginal ultrasound study are at risk for false-positive findings, particularly in the pre-menopausal women, who are an important group to follow, as the median age of familial ovarian cancer patients is only 48 years [16]. Repeat ultrasound examinations, other imaging studies, and their correlation with physical examinations and circulating tumor markers are essential before oophorectomy is recommended in women with endovaginal ultrasound findings compatible with ovarian cancer.

Each participant in the Yale program is encouraged to follow American Cancer Society recommendations for mammography and is requested to have mammogram results forwarded to us at the regularly scheduled intervals. Post-menopausal women are encouraged to have at least one endometrial sampling at the time of entry into the study or at any time thereafter if post-menopausal bleeding develops or if the endovaginal ultrasound examination suggests an endometrial abnormality. Partici-

pants routinely being followed with Pap smears by community physicians are asked to forward the results to us. Pap smears are performed on women who are not undergoing routine Pap smear screening. Participants have stool checked for occult blood at each visit at which a pelvic examination is performed.

All data collected are forwarded to the data coordinating center, Yale University School of Medicine Biostatistics Consulting Unit (YSMBCU), Laboratory of Epidemiology and Public Health. The YSMBCU maintains an on-line inventory of all study patients and forms received to date. A computer program is used to summarize pre-treatment information on patients entered into the study and test results.

Forms are reviewed briefly by data entry personnel for correctness, completeness, and consistency. Data are then keyed on to diskettes, verified, transmitted, and stored on-line. Computer-driven edit checks will be developed to identify missing, out-of-range, and inconsistent values. This program will generate separate error notices for each patient and identify the form in which the errors were noted. A separate file will be kept to contain the data in question and the type of error; this file will be used to edit the necessary data when the requested information is returned. When the errors are corrected, the corrected data will be merged with the analysis data base. A computerized record will be kept of types of errors in order to ensure a high level of data integrity. Multiple copies of the main data files will be extracted periodically from these files for the generation of reports.

Participants will be followed indefinitely as long as they are willing to continue to remain in this project. The Connecticut Tumor Registry has agreed to provide annually the names of all women in Connecticut diagnosed with ovarian cancer. Thus, women who drop out of the study can still be followed with regard to their subsequently developing ovarian cancer. Data will also be analyzed to identify epidemiologic characteristics of those women who might best benefit by undergoing prophylactic oophorectomy.

One of the primary goals of this study is to characterize the usefulness of commercial and investigational tumor tests for use in ovarian cancer screening programs. In addition, we wish to determine the frequency with which such tests should be employed. The overwhelming majority of subjects entering the program will remain ovarian cancer-free throughout the study period, i.e., roughly 98 percent will not develop ovarian cancer. This estimate was derived from two independent sources, the SEER program data tables and Schildkraut and Thompson [14]. Assuming that we will enter 200 subjects per year, we expect to accrue approximately 1,000 high-risk women into our proposed five-year study. This number of subjects will guarantee that our study goals can be addressed. First, we will be able to identify, with a high degree of precision, the proportion of subjects in this well-defined, high-risk population who will develop ovarian cancer. The 95 percent two-sided confidence limit about the observed proportion will be ± 1 percent. In addition, with our extensive collection of baseline data, we will be able effectively to characterize this high-risk population and to define the time to failure precisely (i.e., detection of ovarian cancer) for subjects in this group. The number of subjects also will guarantee that, for certain subgroups of interest (e.g., how many subjects with moderately elevated marker tests who do not go on to develop ovarian cancer), the 95 percent two-sided confidence limits about the observed proportion of subjects who develop ovarian cancer will be small. Moreover, if we observe that the test results are constant over time with limited variability, we can reduce their frequency. This

evaluation will be performed by fitting a regression equation to each subject's values over time and then showing that the slopes of the regression lines hover about a certain value. With this large number of subjects, we will be able to obtain highly precise estimates of the variability of these slopes and thereby identify the stability of test values over time.

The major strength of the Yale program for the early detection of ovarian cancer is that the patient population will be restricted to women at high risk for developing the disease. To our knowledge, this study is a unique program in that all others have concentrated on either post-menopausal women or broad population-based screening. It is believed that the data obtained from the women at high risk for ovarian cancer will be sufficient to make rational screening recommendations for low-risk women as well. Such recommendations do not exist at present. The faculty involved in this program are multi-disciplinary with very strong proven expertise in the diagnosis and management of ovarian cancer. A strong basis for clinical decision making for physicians should be an early outcome of this study. At present, the applications of circulating tumor markers and ultrasound examinations for detection of ovarian cancer are being done in a haphazard fashion by physicians in clinical practice. This program should be able to give firm guidelines for ovarian cancer screening procedures and for responding to abnormal tests resulting from such screening.

ACKNOWLEDGEMENTS

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