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Screening for Ovarian Cancer: What Are the Optimal Surrogate Endpoints for Clinical Trials?

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Abstract The inability to identify relevant markers for presymptomatic screening in early stage or "pre-invasive" ovarian cancer has plagued investigators and clinicians facing the problems of early detection. The characteristic late stage of disease at initial presentation has hindered our understanding of the biologic progression and stepwise molecular alterations that result in ovarian carcinoma. To date, most screening studies have focused on identifying early anatomic changes using ultrasound or fluctuations in serum biomarkers such as CA-125. These screening methodologies have proven inadequate in both sensitivity and specificity for early stage ovarian cancer detection.

Molecular analysis of ovarian carcinomas has revealed alterations in oncogenes and tumor suppressor genes associated with these tumors. The HER-2/*neu* oncogene, a member of the epidermal growth factor family, is amplified or overexpressed in approximately 25–30% of ovarian carcinomas. Significant data substantiate an important role for HER-2/*neu* in the pathophysiology of ovarian cancer. While potentially an attractive surrogate endpoint biomarker (SEB), serum HER-2/*neu* levels have not proven to be a useful screening modality.

In response to the urgent need for improved early detection for ovarian cancer, our current research efforts include differential hybridization studies between normal and malignant ovarian epithelium to define potentially unique ovarian cancer antigens which may ultimately have clinical utility; defining physical alterations that occur in malignant ovarian tissues using implanted telemetry systems; studies using positron emission tomography to detect changes in glucose metabolism between normal and malignant ovarian tissues; and screening studies using a 3-dimensional ultrasound unit to improve the accuracy of this technique in recognizing early neoplastic changes. By taking diverse approaches to tackle this problem, an improved understanding of ovarian carcinogenesis should translate into the identification of appropriate SEBs for early detection.

Key words: Color doppler imaging, HER-2/*neu* oncogene, ovarian cancer screening, ultrasound

Surrogate endpoint biomarkers (SEBs) for ovarian cancer have been difficult to identify, in part due to the biology and late clinical presentation of the disease. The lack of early symptoms has hindered diagnosis prior to metastatic spread, and the intraperitoneal location of the ovaries has hampered the development of effective presymptomatic screening tools. The successes of other screening modalities, such as Pap smear screening for cervical cancer or mammography screening for breast carcinoma [1–4] have been based on both an understanding of tumor biology and on large "screening windows", which provide an opportunity for early diagnosis to translate into improved disease survival. In order to significantly impact ovarian cancer mortality, a true screening test must at a minimum be able to detect asymptomatic Stage I disease, not merely identify already metastatic Stage IIIA

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cases prior to their usual clinical presentation at Stage IIIC. While the doubling time for epithelial ovarian cancers and the time interval between Stage I and Stage III disease remain unknown, recent molecular data have supported a monoclonal origin for most ovarian carcinomas [5,6]. These data at least theoretically substantiate efforts aimed at detecting early neoplastic changes in the ovary. Since little is known about the events leading to ovarian transformation or early ovarian cancer biology, the optimal SEBs remain enigmatic.

ULTRASOUND AND TUMOR BIOMARKERS

To date, most investigators have focused on detecting early anatomic alterations with transvaginal sonography (TVS) and/or fluctuations in shed tumor biomarkers such as CA-125 [7–13]. Since its discovery more than a decade ago, CA-125 has become an important marker used by clinicians to follow the response to therapy for many women with ovarian cancer [14]. CA-125's utility in the screening setting, however, has been disappointing. Initial case reports and retrospective studies noted rises in CA-125 serum levels 6–18 months prior to the clinical presentation of ovarian cancer [15,16]. These encouraging reports led to prospective studies of CA-125's utility in the screening setting. Unfortunately these trials demonstrated that CA-125 lacked both specificity for ovarian cancer as well as sensitivity in diagnosing organ-confined, curable disease [11,12].

Clinical trials utilizing ultrasound techniques for ovarian cancer screening have been underway since the late 1980s [10]. Most investigators have focused on at-risk populations either based on advancing age (postmenopausal) or family histories of ovarian cancer. DePriest and van Nagell [7] reported in their results from annual TVS screening of 3,220 asymptomatic postmenopausal women that 44 (1.4%) morphologic ovarian abnormalities were detected. Surgical exploration of these women revealed two Stage I ovarian cancers (one granulosa cell tumor and one epithelial carcinoma), one Stage IIIB ovarian cancer, and 41 benign ovarian neoplasms. There were 21 serous cystadenomas in this group. These investigators propose a premalignant disposition of these tumors and suggest a potential benefit from their excision. This hypothesis, however, has not yet been validated due to our limited understanding of the precursor lesion or the stepwise progression to ovarian carcinoma development.

Other ovarian cancer early detection studies have coupled conventional ultrasound techniques with color doppler imaging (CDI) in an attempt to visualize early physiologic changes such as angiogenesis prior to detectable morphologic changes in ovarian architecture [10]. A clinical-pathologic study by Granberg *et al.* [17] helps to define the limitations of real time scanning without CDI. These investigators correlated the gross anatomic changes seen in the ovaries at the time of surgery with the specimens' histopathology. Papillary vegetations were the most ominous finding associated with an ovarian carcinoma diagnosis, while cyst size or septations were not correlated with malignancy. The surgeons' prediction of malignancy agreed with the pathologic diagnosis 84% of the time; this number probably represents a realistic estimate of the limitations of ultrasound screening. By coupling this technique with CDI, investigators have tried to improve the sensitivity and accuracy of TVS. Folkman [18] recognized that neovascularization is an obligate early event in tumor growth and neoplasia. CDI attempts to differentiate "malignant" blood flow patterns from physiologic or "benign" neoplastic vasculature. In theory, therefore, this technique could detect neoangiogenesis prior to morphologic changes in ovarian architecture.

Bourne *et al.* [8] used TVS and CDI to screen 1,601 women (ages 17–79) with a family history of ovarian cancer. Nine hundred nine women (57%) required follow-up scans due to initial abnormalities (probably as a result of the large number of premenopausal participants in this study); however, only 61 patients (3.8%) were ultimately felt to have a positive screen and were referred for surgical exploration. Six ovarian cancers (five Stage I, three of which were low malignant potential, and one Stage III) and 48 benign ovarian neoplasms were found. In seven women, no abnormality could be identified at surgery. Over the subsequent 2–4 years of follow-up after the initial ultrasound screen, three additional patients were diagnosed with Stage III ovarian cancers. Two of these cases were felt to be consistent with primary peritoneal carcinomas which may not be amenable to early detection with TVS. The late stage of disease at the time of di-

agnosis in these two cases highlights our ignorance about the time interval required to develop metastatic ovarian cancer. These investigators conclude that ovarian cancer screening should be performed at least more frequently than every two years.

Our early detection studies have also focused on women with a family history of cancer [9]. We screened study participants biannually with TVS, CDI, and five tumor biomarkers: CA-125, HER-2, UGP, DM70K, and LASA. Since July, 1991, 1,079 women (ages 35–80) have undergone 3,603 screens. Persistent abnormal test results have resulted in 34 women undergoing bilateral oophorectomies, while another 37 women underwent elective surgery following genetic counselling. One Stage IA and one Stage IB ovarian cancer (both low malignant potential) and one Stage IA Grade 3 endometrial cancer were identified by TVS screening. No consistent pattern of ovarian pathology was discerned from review of all the surgical specimens. One additional patient presented with abdominal symptomatology four months following normal screening results and was found to have Stage IIIC primary peritoneal carcinoma. Her CA-125 values doubled from 11 U/ml to 22 U/ml during her last six months in our study. While these values are in the normal range, some investigators have suggested improving the specificity of CA-125 by looking at its rate of rise [13]. Using mathematical modelling, Skates and Singer [19] estimated that the rate of increase in CA-125 correlated with tumor growth. Only two other patients in our study demonstrated a doubling of CA-125 values over a six-month interval. These women remain disease-free. It is possible that introducing a time element into the "biomarker equation" for ovarian cancer detection may improve the specificity of these tests.

Statistical analysis of our biomarker data show significant differences between each of the median premenopausal and postmenopausal biomarker values [20]. CA-125 was the only marker consistently higher in the premenopausal population. All other markers' median values were higher in postmenopausal women. A single elevated biomarker value was detected in the absence of ovarian cancer in up to 18% of the premenopausal women. When the tests were repeated, approximately 80% of initially elevated values for LASA, DM70K and UGP fell to normal levels at the second screen. Notably, 69% of elevated CA-125 values in premenopausal women remained elevated. Frequent elevations of LASA and UGP were detected in up to 25% of postmenopausal participants; however, approximately 80% of the initially elevated values returned to normal levels on follow-up screens.

MOLECULAR MARKERS

While a stepwise progression of genetic alterations leading to ovarian carcinogenesis has not been defined, mutations in oncogenes and tumor suppressor genes associated with ovarian cancer have been extensively studied. The dysregulated expression of growth factors, their receptors, or their signalling pathways are some of the most frequent changes implicated in ovarian carcinogenesis [21]. Among these, alterations in members of the epidermal growth factor (EGF, c-*erb*B) receptor family have been associated with ovarian cancers. The HER-2/*neu* proto-oncogene (c-*erb*B-2) encodes a 185 kDa transmembrane tyrosine kinase protein $p185^{HER\text{-}2/neu}$ with homology to the EGF receptor [22,23]. The oncogenic potential of HER-2/*neu* may be activated by a point mutation in the transmembrane domain, truncation of the extracellular domain, or overexpression of the non-mutated protooncogene [23–26] Substantial data suggest a direct role for the HER-2/*neu* oncogene in the pathogenesis of ovarian cancer. Molecular analysis of ovarian carcinomas revealed that 25–30% of tumors demonstrate HER-2/*neu* amplification or overexpression. In these patients, HER-2/*neu* overexpression is correlated with a shorter survival and poor response to therapy [27,28]. While $p185^{HER\text{-}2/neu}$ overexpression may merely represent a tumor marker useful in predicting disease outcome, other studies point to a more intricate role for the HER-2/*neu* growth factor receptor in ovarian carcinogenesis. A point mutation in the transmembrane domain of the rat *neu* gene was shown to increase tyrosine kinase activity of the altered $p185^{HER\text{-}2/neu}$ receptor [23]. This enhanced signalling activity is believed to be responsible for the ability of the mutated gene to transform NIH/3T3 cells and cause breast carcinomas in transgenic mice bearing *neu* [24,29]. Human tumors examined thus far, however, have not demonstrated an analogous point mutation. Rather, amplification and protein overexpression of the

normal HER-2/*neu* gene is consistently observed [27,28,30]. Overexpression of the normal human HER-2/*neu* gene in NIH/3T3 cells or immortalized (but not transformed) human breast cells resulted in transformation of these cells [24,31]. In addition, established breast and ovarian carcinoma cell lines engineered to overexpress HER-2/*neu* were shown to have enhanced cell growth, DNA synthesis, anchorage-independent growth, and tumorigenesis in nude mice compared with the appropriate control cells [32]. Regardless, normal ovarian epithelial cells similarly engineered to overexpress HER-2/*neu* are not immortalized or transformed by this genetic alteration alone (unpublished results). This finding is not surprising, however, since most normal adult tissues must accumulate a series of genetic alterations in the transformation process [33]. Nonetheless, significant evidence supports a potential pathogenic role of HER-2/*neu* in ovarian epithelial neoplasia, and HER-2/*neu* may yet prove to be a useful candidate SEB for ovarian cancer.

Assays to detect the extracellular domain of the HER-2/*neu* antigen in patients' sera have been developed. As outlined earlier, we have investigated HER-2/*neu* as a tumor biomarker in the screening setting [9,20]. Asymptomatic women with a family history of cancer participating in our multimodality ovarian cancer screening program were found to have elevated serum levels of HER-2/*neu* in 4% of the premenopausal and 4% of the postmenopausal populations. These numbers may be compared to CA-125 biomarker values in these same patients; 18% of the premenopausal and 2% of the postmenopausal participants had elevated CA-125 levels. None of these women have subsequently developed cancer. The higher median values of HER-2/*neu* and the higher prevalence of elevated values seen in the postmenopausal women may reflect the accumulation of genetic alterations that occurs with aging. Our pilot study of 51 ovarian cancer patients revealed that 2/19 patients with HER-2/*neu* overexpressing tumors had elevated serum values, while none of the 32 patients whose tumors were HER-2/*neu*-negative had detectable serum elevations of HER-2/*neu*-antigen (unpublished results). McKenzie and colleagues [34] studied 48 ovarian cancer patients and found 5 out of 17 women with HER-2/*neu* overexpressing tumors had elevated levels of HER-2/*neu* in the serum, while only 2 out of 28 patients with normal tumor HER-2/*neu* expression demonstrated elevated serum levels. Although HER-2/*neu* may play a role in ovarian cancer biology, at the current time other SEBs must also be investigated.

NEW DIRECTIONS

Ongoing investigations in our laboratory and others are aimed at identifying molecular changes associated with ovarian epithelial transformation which may yield useful SEBs for ovarian cancer early detection. To this end, we have begun to search for novel ovarian cancer antigens by means of differential hybridization of cDNA libraries. Our laboratory has ready access to both normal and malignant ovarian epithelia, and we have developed techniques to propagate these tissues *in vitro* [35,36]. In collaboration with Dr. Leroy Hood's laboratory, more than 5,000 clones have already been isolated from cDNA libraries constructed from these tissues. These clones are currently being screened for ovary-specific and ovarian cancer-specific sequences, as well as genes with altered expression levels in ovarian tumors. The vector chosen for constructing these cDNA libraries will also support expression in a eukaryotic system. Thereby, it is possible to translate the respective proteins and raise antibodies against them. We will then be poised to bring these studies to the clinic, for example, through antibody-based detection of the newly identified circulating tumor antigens. Another approach may be the development of sensitive peripheral blood-based reverse transcriptase-polymerase chain reaction testing to detect novel molecular alterations in peripherally circulating tumor cells. This approach has recently been reported to offer greater sensitivity than prostate specific antigen-based assays for detecting organ-confined, curable prostate cancer [37]. Targets for potential therapeutic interventions are also likely to be identified by these studies.

While new molecular tools will allow us to more precisely define ovarian carcinogenesis, other physical and chemical properties of ovarian cancer cells may also hold promise as SEBs. In collaboration with scientists at the Jet Propulsion Laboratories (JPL), we are investigating clinical applications of implantable telemetry systems which incorporate high resolution pixel cameras.

These cameras, initially developed by NASA/JPL for planetary investigations, will contain a laser light source and be augmented by acoustic-optical filter technology and solid-state mirrors. The signals will be transmitted by a JPL micropowered telemetry system to an external PC for viewing and processing. The data will be co-registered to determine a pixel's ultrasonic, infrared, and radio-frequency absorption, and/or its color and temperature spectra. A computer algorithm would then be generated to best differentiate normal from malignant tissues.

In addition, we are studying early biochemical changes in ovarian cancer cells, such as their characteristic increased metabolic rate, by means of positron emission tomography (PET). Using (fluorine-18)-2-deoxyglucose-PET, we have successfully visualized primary and metastatic ovarian cancers based on their increased glucose metabolic rate [38,39]. These preliminary studies have not as yet demonstrated adequate sensitivity to diagnose early ovarian cancers. Recently, we have begun testing a 3-dimensional ultrasound unit to provide a hologram-like image of the ovary. While these advances in ovarian visualization may improve our predictive accuracy in predicting malignancy, this modality is still limited by the requirement for anatomic changes on the ovarian surface.

In 1994, the NIH Consensus Panel on ovarian cancer screening, treatment, and follow-up concluded that "there is no evidence available yet that the current screening modalities of CA-125 and transvaginal sonography can be effectively used to reduce the mortality of ovarian cancer nor that their use will result in decreased rather than increased morbidity and mortality" [40].

While data on these screening modalities clearly support the panel's statement, continued investigations aimed at expanding our current understanding of the tissue, cellular, and molecular alterations associated with ovarian carcinogenesis hold promise to reveal yet untested means of early ovarian cancer diagnosis.

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