

CA 125: History, Current Status, and Future Prospects

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DEVELOPING THE CA 125 ASSAY

The development of an assay for the CA 125 tumor marker grew out of attempts to obtain monoclonal reagents for serotherapy of patients with ovarian cancer. In the early 1970s, it was discovered in an animal model that the intraperitoneal injection of a heteroantiserum developed in rabbits against a partially purified tumor-associated antigen could inhibit the intraperitoneal growth of syngeneic murine ovarian cancer transplants (1,2). Soon after, it was found that the antitumor activity of heteroantiserum could be augmented by coinjection of the immunostimulant *Corynebacterium parvum* (3), which was shown to attract and activate inflammatory cells capable of mediating antibody-dependent cell-mediated cytotoxicity (4). By the early 1980's, clinical studies using intraperitoneal injection of *C. parvum* as a single agent demonstrated the objective regression of small amounts of ovarian cancer in patients with persistent or recurrent disease (5).

With the discovery of the monoclonal technology by Kohler and Milstein in 1975 (6), the present authors attempted to develop antibodies reactive with ovarian cancer that might be administered in combination with *C. Parvum*. Mice were repeatedly injected with a human ovarian cancer cell line and hybridomas were prepared from immune spleen cells and the P3NS-1 plasmacytoma. From these hybridomas, clones were isolated based on the production of antibodies that bound to the ovarian cancer cell line used for immunization, but not to a B lymphocyte cell line developed from the tumor cell donor. The one hundred twenty-fifth promising clone produced an IgG1 antibody of the desired specificity and was designated OC (ovarian cancer) 125.

Using immunohistochemical analysis, the OC 125 antibody was found to bind antigen expressed by approximately 80% of epithelial ovarian cancers, as well as by other gynecologic, breast, lung, and colon carcinomas; this antigen was designated CA (cancer antigen) 125 (7). Mucinous ovarian cystadenocarcinomas were less frequently positive than serous and endometrioid tumors. In addition, OC 125 reacted with amnion and coelomic epithelium in embryos, as well as with a fraction of secretory endometria in adults.

Although OC 125 was not of an appropriate isotype to interact with human effector cells and was of little value for serotherapy, the antibody did prove useful as a diagnostic reagent. Working with Drs. Vincent

Zurawski and Thomas Klug, the authors utilized the OC 125 antibody to develop a double determinant radioimmunoassay for the detection of CA 125 antigen that had been shed into serum and body fluids (8). In a protocol exploiting the multiplicity of CA 125 determinants expressed on a single molecule, OC 125 antibody fixed to nitrocellulose beads was used to trap antigen from the fluid phase, and additional OC 125 labeled with ^{125}I was then used as a probe to detect antigen that had been trapped. This culminated in the development of the first monoclonal antibody radioimmunoassay for the monitoring of epithelial ovarian cancer, reported in 1983 in the *New England Journal of Medicine*. Since the development of the original methodology, several different formats for the assay have been developed using radiolabeled or enzyme-labeled OC 125 as a probe. Although the correlation among assays has been acceptable, the variation among methods is sufficiently great that the use of a single assay is recommended to monitor individual patients over time.

CHARACTERIZATION OF CA 125

Progress toward the characterization of CA 125 began in the mid-1980s. CA 125 determinants were found to be associated with a family of high molecular weight glycoproteins, that differed from classical mucins by means of carbohydrate conversion (less than 50%) and the presence of both N- and O-linked carbohydrate residues (9). The smallest subunit retaining reactivity with OC 125 exhibited a mass of approximately 220 kD.

Chemical study has revealed sensitivity of CA 125 to proteases, low pH, high temperature, and high ionic strength, properties consistent with a conformational peptide determinant. However, CA 125 activity can also be destroyed with relatively high concentrations of periodate and blocked with different lectins, suggesting a close association with carbohydrate. Despite these observations, the chemical composition of the CA 125 determinant is still not completely defined.

Furthermore, despite repeated attempts to clone the CA 125-associated protein, the isolation of an appropriate gene has not yet been reported. Using a polyclonal heteroantiserum, a gene was isolated from a bacterial expression library that mapped to the region of BRCA1 (10). The product of this gene does not, however, appear to react with OC 125.

Over the last decade, a number of monoclonal antibodies have been developed that react with one or two distinct epitopes on molecules expressing CA 125 (11). One of these antibodies, M11, has permitted the development of a second generation assay, CA 125-II, in which M11 is used to trap antigen, followed by OC 125 to detect antigen that has been captured on a solid phase.

CLINICAL APPLICATIONS

The CA 125 assay was originally developed to monitor the course of patients with epithelial ovarian cancer. After cytoreductive surgery, small, non-palpable nodules of residual disease below the limits of resolution for computerized tomography and magnetic resonance imaging often remain within the peritoneal cavity. Cytotoxic chemotherapy is often administered for many months without a clear indication of whether the disease is responding to treatment. If a cutoff of 35 U/ml is chosen, which excludes 95% of apparently healthy individuals, more than 80% of patients with clinically evident ovarian cancer will have elevated CA 125 levels (8). In patients with early stage disease and in those mucinous tumors, a smaller percentage exhibit elevated antigen levels.

It has been shown that CA 125 is not a specific marker for ovarian cancer in that it can be elevated in sera from patients with other gynecological adenocarcinomas, as well as lymphomas, malignant mesotheliomas, immature teratomas, and carcinomas of the pancreas, colon, breast and lung. Elevations were also observed with benign conditions including severe endometriosis, adenomyosis, uterine fibroids, ovarian cysts,

salpingitis, peritonitis, pleuritis, pericarditis, alcoholic hepatitis, and the first trimester of normal pregnancy. Although many of these conditions might complicate use of CA 125 for screening a general population, they can be readily excluded when monitoring patients with known ovarian cancer.

A persistently rising level of CA 125 has been associated with progressive growth of ovarian cancer in more than 90% of instances studied (12). Decreasing levels of CA 125 have correlated with decreases in the volume of disease sufficiently often that CA 125 has been proposed as an appropriate surrogate for measurable disease in conducting phase II trials of new drugs in patients with ovarian cancer (13). CA 125 can, however, return to less than 35 U/mL prior to "second look" operations, and small volumes of disease can still be found in approximately half of patients. Consequently, CA 125 is not optimally sensitive as a marker for residual disease, but, if elevated, can predict the persistence of disease with greater than 95% accuracy (12). This correlation prompted approval of the CA 125 assay by the United States Food and Drug Administration.

In monitoring patients who have responded completely to cytoreductive surgery and chemotherapy, progressive elevation of CA 125 has preceded clinical detection of disease recurrence by an average of 3 months in many, but not all, studies. Doubling of initially elevated CA 125 levels has been associated with disease progression in more than 90% of cases (12), although false positive elevations can occur and documentation of initial recurrence by an additional imaging modality and biopsy is desirable. Lack of effective salvage therapy, however, has limited the value of sequential monitoring for disease recurrence. Critical studies are needed to establish whether the small volumes of disease that can be detected with the aid of CA 125 are more responsive to additional treatment, and whether this translates into longer survival and/or a better quality of life. As more effective treatment becomes available for previously treated ovarian cancer, the use of CA 125 and other markers for monitoring will attain greater importance.

CA 125 levels at the time of diagnosis have not correlated consistently with prognosis, but numerous studies now suggest that the rate at which CA 125 declines during chemotherapy can predict the probability of finding disease at "second look" surveillance procedures and correlate with survival. The actual half life of CA 125 is biphasic, with an average of 4.5 days. Apparent half-lives during chemotherapy in excess of twenty days have been associated with a poor prognosis, presumably related to the persistence of tumor that sheds CA 125 (14). Computer-based analysis has produced more precise discriminants of tumor response.

CA 125 can be used to distinguish malignant from benign pelvic masses. Even in postmenopausal patients the majority of pelvic masses are found to be benign; nevertheless, surgical exploration is still often required to rule out malignancy. Given the value of careful staging and cytoreductive surgery, primary exploration in those cases which are malignant could best be carried out by surgeons who have had extensive experience in treating ovarian cancer. In this context, use of CA 125 could permit triage of patients judged to be at high risk of malignancy. Among postmenopausal patients with a pelvic mass, a CA 125 level greater than 65 U/mL has distinguished malignant disease with greater than 90% accuracy. More complex indices have been developed which incorporate the results of sonographic evaluation (15). As ovarian cancer can be found in the presence of a normal CA 125, a value less than 35 U/mL should never discourage surgical exploration of a postmenopausal patient with a suspicious adnexal mass. The use of ultrasound and multiple serum markers has, however, improved the negative predictive value of a normal CA 125 in premenopausal patients. Further development of appropriate indices may encourage serial observation and spare patients unnecessary surgery.

Possibly the most important anticipated role for CA 125 is as one component of a strategy for early detection of ovarian cancer. If cancer is still limited to the ovaries at the time of diagnosis, more than 90% of patients can be cured with conventional surgery, radiotherapy or chemotherapy. More than 70% of ovarian cancers are diagnosed in advanced stages, where only 20% of patients can be cured. Ovarian cancer has been shown to be a clonal disease in approximately 90% of cases (16,17), suggesting that most cancers could, in fact, be detected before they have metastasized. Given the prevalence of ovarian cancer among postmenopausal

women in North America, an effective screening strategy must have high specificity. To detect 75% of ovarian cancers with a positive predictive value of 10% (equivalent to the diagnosis of one case of ovarian cancer per 10 laparotomies performed), a specificity of 99.6% must be attained.

At present there is no proven strategy for early detection of ovarian cancer. Annual pelvic examination is notoriously insensitive. Transvaginal sonography (TVS) is capable of detecting more than 95% of stage I ovarian cancers, but also detects large numbers of patients with benign disease who subsequently undergo surgery to rule out malignancy. Among 11,283 women screened with TVS, 486 laparotomies were performed to detect 13 cancers, of which eight were borderline and only five were frankly invasive (18). Thus 97 laparotomies were performed for each case of invasive ovarian cancer detected. In addition, the current cost of TVS would be prohibitive if extended to every postmenopausal woman every year, as a screening test.

Elevations of CA 125 have been documented 10 to 60 months prior to the clinical detection of primary epithelial ovarian cancer (19-21). Using a single determination of CA 125, the assay is not optimally sensitive at a threshold of 30 or 35 U/mL. CA 125 attains this level in approximately 50% of patients with stage I disease (12). Even with relatively high thresholds, the specificity of a single CA 125 determination does not exceed 99%, but can be improved through the subsequent use of sonography and by sequential monitoring of CA 125. A pilot trial of 4000 women in the United Kingdom conducted by Jacobs and colleagues found that a combination of CA 125 and transabdominal ultrasound achieved an overall specificity of 99.9% (22). In a subsequent screening trial of 22,000 postmenopausal women, a CA 125 greater than 30 U/mL was found in 340 (23). Ultrasound was abnormal in 40 of these individuals who then underwent laparotomy leading to the diagnosis of 11 ovarian cancers. Over the next year, three additional cases of ovarian cancer were detected among women who had a single normal CA 125 determination at the time of screening, resulting in a sensitivity of 78% (11/14) for CA 125 measurements taken within one year of diagnosis. This is the most appropriate estimate of sensitivity, if one considers annual CA 125 measurements to be the screening program of choice. An additional five cases of ovarian cancer were detected between one and two years after their initial CA 125 measurement.

Measuring CA 125 over time can also improve the specificity of the assay (24). In a trial of 5,550 women in the Stockholm area conducted by Einhorn and Sjøvall, abnormal CA 125 (> 30 - 35 U/mL) was found in only 2% of subjects over age 50 (25). Individuals with abnormal CA 125 were followed quarterly with CA 125 determinations and semiannually with pelvic examinations and ultrasounds. Laparotomies were performed in 21 women, detecting six ovarian cancers with four in stage I or stage II. Subsequently, three additional cases of ovarian cancer were detected among women who had a single CA 125 determination with a level less than 30-35 U/mL, all of whom were under 50 years of age.

Using serum samples from the Stockholm study and the second generation CA 125-II assay, an algorithm has been developed with Stephen Skates that considers both the slope and intercept of CA 125 values over time (26). A risk of ovarian cancer (ROC) can be assigned with this algorithm that achieves a specificity of 99.7% and a positive predictive value of 16%. The apparent sensitivity of 83% in the study, with the limited number of cancer cases, does not give a precise estimate of sensitivity. When using the ROC algorithm, the highest level of CA 125 that does not require additional determination before the next annual test is approximately 15 U/mL as compared to previously used values. This lower cutoff level for subsequent action potentially improves the sensitivity of the overall program. The increased sensitivity may not, however, exceed 80% for early stage disease.

It is apparent from immunohistochemical studies that between 10% and 20% of ovarian cancers fail to express CA 125 determinants. Consequently, other serum markers have been evaluated to determine whether a combination of markers might achieve greater sensitivity without sacrificing specificity. The most promising combination of markers evaluated to date has included CA 125, macrophage colony stimulating factor (M-CSF or CSF-1) (27), and a mucin marker OVX-1 (28). In a retrospective analysis, one of the three

markers was elevated in 98% of 46 sera from patients with well-documented stage I disease (29). Among 200 postmenopausal women with CA 125 less than 35 U/mL, the OVX-1 or M-CSF levels were elevated in 11%. While a specificity of less than 89% is not adequate to prompt surgical intervention, abnormal blood tests could trigger transvaginal sonography. In this context, combination with multiple markers should also improve the specificity of TVS. The three markers distinguish malignant from benign pelvic masses with a specificity of 50%, reducing the number of false positive studies on subsequent ultrasound examination. At present, a trial being designed in the United Kingdom will utilize the ROC determined by sequential CA 125-II values, potentially supplemented with OVX-1, to prompt TVS. More than 100,000 women over the age of 50 will be randomized to this form of screening or to routine surveillance, with the expectation of studying survival as an end point.

Urban et al. have recently utilized a stochastic simulation model to identify optimal screening strategies (30). The use of a rising or elevated CA 125 to trigger TVS was found to be the most efficient strategy in that no single modality saved as many years of life at lower cost per year of life saved. Used annually, this strategy cost less than \$100,000 per year of life saved, which compares favorably to several other widely applied interventions, such as mammography and the detection and treatment of hypertension.

FUTURE PROSPECTS

If the currently available serum CA 125 assays prove inadequate, new technologies can be applied to detecting additional markers for ovarian cancer in the future. For example, recent use of two-dimensional gel electrophoresis has led to the detection of a 30 kd protein of pI 6.1 that is shed by ovarian cancer cells but not by normal ovarian epithelial cells (31). Likewise, differential display of expressed genes has detected fragments that are expressed by ovarian cancers but not by normal epithelial cells (32). Optimal use of multiple markers has been facilitated by classification and regression tree (CART) analysis that promises to improve both the specificity and sensitivity when using multiple tests in combination (33). The serial behavior of multiple markers over time could further enhance sensitivity and specificity when incorporated into an expanded ROC algorithm. Benign causes of elevation in any of these markers are likely to produce a flat slope over time, in marked contrast to the exponential increases over time that reflect the doubling time of malignancy.

Should these newer strategies prove sufficiently sensitive and specific, future efforts will be required to minimize the cost of their use in screening large populations.

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BIOGRAPHY

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