Tumor Markers in Ovarian Malignancies

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ABSTRACT

Epithelial ovarian cancer is the most common ovarian malignancy. CA125, the glycoprotein defined by the antibody OC 125, is the most important clinical marker for the diagnosis, treatment and follow-up of epithelial ovarian cancer. However, like most tumor markers, it is neither wholly specific nor sensitive for the disease. We discuss how CA125 in combination with other tests can be used in the differential diagnosis of pelvic masses and as part of the investigations for cancer screening. CA125 is an important indicator of response to treatment, guiding therapeutic decisions and identifying those patients whose response to chemotherapy and survival is short. CA125 has recently been shown to correlate well with response and can be used to define relapse. Thus, it can be used as a surrogate endpoint in the assessment of new therapeutic modalities as well as in reducing the need for tumor imaging. At the moment, the other tumor markers for non-germ-cell neoplasms of the ovary are clinically less important than CA125, but their role alone or in association with CA125 is the subject of intense study as the search for ideal tumor markers to identify early disease, prognosis, and relapse continues. The Oncologist 1997;2:324-329

INTRODUCTION

Malignant epithelial ovarian tumors account for 90% of all malignancies of the ovary and are the fourth most common cause of tumor-related death in women. The empirical lifetime risk of developing ovarian cancer is 1:70 [1], and most women present with advanced disease (FIGO stage III or stage IV), which is rarely curable. Tumor-associated antigens released into the circulation have been described in many diseases. Ideally, a tumor marker should be able to detect subclinical disease (i.e., screening), useful in monitoring the response to treatment, and to identify early recurrence so that further treatment can be instituted. Furthermore, the release of circulating tumor antigen provides an identifiable surface target on the tumor cell that might be used for in vivo diagnosis or antigen-directed therapy. No serum tumor marker, with the possible exception of human β chorionic gonadotrophin, meets all these criteria. Nevertheless, measurement of many serum tumor markers has been incorporated into clinical practice. This review will focus on CA125, the most clinically applicable tumor marker for ovarian cancer, and will briefly describe other tumor markers and possible future applications of tumor markers.

Bast and colleagues in 1981 first described CA125, a 200 kd glycoprotein recognized by the murine monoclonal antibody OC 125 as a marker for epithelial malignancies [2]. A raised level of antigen was detectable in the serum of 82% of women with epithelial ovarian cancer but in only 1% of healthy blood donors [3]. Epithelial ovarian cancers with low or normal levels of CA125 are usually mucinous tumors. The antigen is not specific to ovarian cancer as raised serum levels may also be found in 29% of other cancers (lung, breast, pancreas, and colorectum) and in 6% of women with nonmalignant conditions such as cirrhosis with ascites, acute pancreatitis, ovarian cysts, endometriosis, and pelvic inflammatory disease.

Screening and Diagnosis

As early-stage ovarian cancer carries a much more favorable prognosis, there is an urgent need to identify subclinical disease. A satisfactory method of screening subclinical disease for ovarian cancer is needed (Table 1). Serological markers are theoretically an ideal approach but none have 100% specificity and sensitivity. In a retrospective study of stored sera from the JANUS serum bank in Norway, raised CA125 levels were found in half of the samples collected within 18 months of the diagnosis of ovarian cancer, and approximately 25% of samples had raised serum CA125 levels within 60 months preceding the diagnosis of ovarian cancer [4]. In a review of the literature, Jacobs and Bast [5] found that about 50% of patients with stage I disease had elevated levels of CA125. However, even in the...
The use of tumor markers to monitor response to treatment is particularly helpful in ovarian cancer where there is often a lack of clinically or radiologically measurable disease. A reduction in the serum CA125 level correlates well with clinical response. Failure of CA125 to fall with chemotherapy indicates drug resistance and identifies a need to change treatment (Fig. 1). Formal CA125 response criteria have been formulated (Table 3) [14]. These parameters were tested on 629 of 989 patients considered assessable for response according to serum CA125 levels in three trials (North Thames Ovary Trials 3 and 4, Gynecologic Oncology Group [GOG] protocol #97). The tumor response rate was 66% in patients assessable by CA125 in GOG #97 and 62% by the GOG-defined response rate. The response rate in patients where CA125 was not assessable was 67%. The specificity was very high and the sensitivity was 68% [14]. It should be noted that removal of ascites will interfere with the serum CA125 level.

Relapse

It has been accepted for a long time that a rise in CA125 into the abnormal range is highly predictive for relapse [3, 15]. However, the lead time to clinical relapse is variable and a clearer definition of relapse is needed if CA125 measurement is to be used as a definition of clinical progression, particularly in the context of a clinical trial. In a recent analysis of 255 patients from a North Thames Ovary Trial, progressive disease was defined as a doubling of the CA125 from the upper limit of normal (30 U/ml “cut-off” in this study). The sensitivity was 85.9% and the specificity was 91.3%, giving a positive predictive value of 94.8%. If a confirmatory elevated CA125 was obtained, the false positive rate fell to less than 2%, with only a small fall in the sensitivity. The median lead time to clinical progression was 63 days [16].

Table 1. Screening for ovarian cancer

| A. Identify preclinical/early stage I disease          | Prognosis similar to clinical stage I disease? |
| B. High prevalence                                    | Who should be screened?                        |
| C. Validity                                           | High sensitivity and specificity?              |
| D. Screening test                                      | Positive predictive value \( \geq 10\% \) (i.e., 1 out of 10 diagnostic laparotomies positive)? |

The presence of an ultrasonographically defined mass, serum CA125 cannot reliably distinguish between a malignant or benign mass. CA125 levels were raised above 35 U/ml in 78% of women with malignant masses, but also in 22% of those with benign masses [6]. The predictive value of CA125 measurement in postmenopausal women is a little greater, and using a “cut-off” of 65 U/ml, the false positive rate was about 8% [7].

Population screening with ultrasonography alone has not proved to be a cost-effective means of detecting ovarian cancer. However, the sensitivity and specificity of this investigation can be increased by transvaginal ultrasonography and transvaginal color doppler imaging. Serum CA125 measurement in healthy women has been used as a means of selecting women for ultrasonography. This increases the specificity of examination, but the predictive value of screening is about 10% [8]. At this level, a significant number of surgical explorations would be performed for nonmalignant ovarian pathology. Furthermore, it is not clear how often patients should have examinations repeated, as some of the patients with normal CA125 at screening subsequently developed ovarian cancer on follow-up [9]. Currently, the combination of CA125 and transvaginal color doppler studies is likely to be the most successful screening tool, particularly if applied to women with a strong family history of ovarian cancer, as they have a higher risk of developing the disease. The role of screening in the general population is unclear but is now being tested in a large randomized trial in the UK using the criteria illustrated in Table 2.

The inclusion of other tumor markers may further increase the specificity of screening. Einhorn et al. evaluated CA125 concentrations together with those of CA15-3 and TAG-72 in 219 patients undergoing diagnostic laparotomy for pelvic masses. They found that the three tumor markers increased the specificity for detecting ovarian cancer but reduced the sensitivity of the CA125 assay [10]. Similar observations were made by Soper et al., who showed that by combining CA15-3 and TAG-72 measurements with CA125, the specificity for detecting ovarian cancer rose from 83% to 98%, but the sensitivity was reduced from 88% to 73% [11]. Several other serological markers have been described in association with ovarian cancer. None have the same level of sensitivity and specificity as CA125. As some have a higher specificity, they may be used in combination with CA125 although the sensitivity would be sacrificed. In stage I disease where serum CA125 has a low sensitivity, there may be scope to increase the sensitivity of screening by using a combination of tumor markers. Preliminary reports with CA125, OVX1, and M-CSF are encouraging; 98% of sera from 46 patients with stage I disease had elevated levels of one of these serological markers [12]. A D-dimer of CA125 has recently been analyzed in 56 patients with epithelial ovarian cancer and 65 women with benign ovarian disease. All women in whom the CA125 level was >65 U/ml and the D-dimer level was >416 ng/ml had ovarian cancer (specificity and positive predictive value of 100% with a sensitivity of 73%) [13].

Monitoring Response to Therapy

A reduction in the serum CA125 level correlates well with clinical response. Failure of CA125 to fall with chemotherapy indicates drug resistance and identifies a need to change treatment (Fig. 1). Formal CA125 response criteria have been formulated (Table 3) [14]. These parameters were tested on 629 of 989 patients considered assessable for response according to serum CA125 levels in three trials (North Thames Ovary Trials 3 and 4, Gynecologic Oncology Group [GOG] protocol #97). The tumor response rate was 66% in patients assessable by CA125 in GOG #97 and 62% by the GOG-defined response rate. The response rate in patients where CA125 was not assessable was 67%. The specificity was very high and the sensitivity was 68% [14]. It should be noted that removal of ascites will interfere with the serum CA125 level.

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Measurements of CA125 are frequently taken after the completion of chemotherapy, outside clinical trials. From the results of Rustin et al., the predictive power of CA125 follow-up certainly reduces the need for regular abdominopelvic scans [16]. However, while normal levels of CA125 are reassuring for the patient and her doctor, their measurement often evokes a period of anxiety. Furthermore, there are no clear guidelines to follow during the period between CA125 relapse and clinical progression of disease. We do not know whether the institution of second-line therapy at the time of CA125 (subclinical) relapse is preferable to waiting until clinical relapse has occurred. The Medical Research Council (MRC) Gynaecological Cancer Working Party and the European Organisation for Research and Treatment of Cancer (EORTC) Gynaecological Cancer Cooperative Group have recently started randomized trials to determine whether there is any benefit in survival and quality of life from the early introduction of chemotherapy based on CA125 relapse. Although these trials address important scientific and health-economic issues, recruitment could be slow as clinicians and patients may feel uncomfortable about not knowing the results of tests that have been taken.

In approximately 20% of patients, serum CA125 levels are not elevated. The majority of these patients have mucinous tumors. In such cases, other tumor markers, such as carcinoembryonic antigen or TAG-72, which are more often elevated in mucinous tumors, may be used. The clinician will need to depend more on CT imaging in cases where serological markers are not detectable.

### Prognostic Marker

It would be helpful if reliable prognostic indicators for survival could be determined before treatment. This is particularly true for stage I disease where there is doubt about the need for adjuvant therapy. For more advanced tumors, the value of prognostic markers is less clear as there are fewer options for therapy at the moment. Preoperative CA125 does not appear to be an independent risk factor for survival, although a multivariate analysis of 201 patients with stage I disease concluded that the preoperative CA125 level was the most powerful prognostic factor for survival [17]. In addition, it does reflect a larger tumor burden and more advanced stage [18]. Serum CA125 levels may rise shortly after surgery and even in stage...
I disease may not return to normal until four weeks after laparotomy. A raised level of CA125 after this time has prognostic value as the patient has persistent disease and does not have a true stage I tumor. In a collaborative study from the Gynaecological Tumour Marker Group in Germany, it was reported that the postoperative value of CA125 was a predictor for survival. All patients with less than 2 cm residual disease who had a CA125 >65 U/ml died within 42 months, whereas 48% of those whose level was less than this were alive at six years [19].

The rate of fall of CA125 in response to chemotherapy, particularly during the early period of treatment, does provide prognostic information. Mogensen reported that if the titer of CA125 was ≤10 U/ml after three cycles of treatment, the median survival was 60 months. If it was ≥100 U/ml, the median survival was seven months [20]. Similar findings have been reported by others [21-24]. The MRC Working Party on Gynaecological Cancer analyzed 248 patients from 11 centers and found that the absolute value of the third serum CA125 level was the most important factor for predicting progressive disease at 12 months, with the addition of residual bulk disease at the end of initial surgery slightly improving the predictive power; however, there was still a false positive rate of 20% [25].

“Second-look” procedures are now uncommonly performed, but the prognosis is significantly worse in patients with raised CA125 levels at the time of surgery. CA125 is generally considered to be an insensitive predictor of laparotomy findings [26], although others have suggested that it is an independent prognostic factor [27]. It has been suggested that the sensitivity of predicting disease can be increased by combining the measurement of CA125 with the OVX1 assay, which detects an epitope on a high molecular weight mucin molecule. The prediction of a positive second-look laparotomy could be increased from 35% with CA125 alone to 56% [28].

Other Tumor Markers

The use of other tumor markers in epithelial ovarian cancer has been briefly discussed above and listed in Table 4. None so far are being used as frequently as CA125 measurement in clinical practice. Levels of sialyl Tn, an antigen of the core region of mucin oligosaccharide, are raised in the preoperative serum of almost half the patients with ovarian cancer, and raised levels are an adverse prognostic determinant [29]. Various cytokines, including M-CSF, GM-CSF, IL-1, IL-6, and TNF-α are produced by ovarian cancer cells. Both M-CSF and its receptor fms can be expressed by ovarian cancer cells, and the levels of fms have been demonstrated to correlate with both advanced histological grade and clinical stage and are therefore associated with a poor clinical outcome [30, 31]. IL-6 is produced by ovarian cancer cells and can be isolated from the ascitic fluid and serum of patients. In one study, there was a correlation between an elevation of the serum level of IL-6 and disease extent with a raised IL-6 in 76% of patients with macroscopic disease, but only 13% in those with microscopic disease and 17% in healthy controls [32]. The IL-6 produced by such tumor cells, however, is not distinguishable from that produced by cells of the immune system, making it a nonspecific marker [33]. Furthermore, cytokine production may not arise from tumor cells. IL-10, a cytokine with various immunoinhibitory functions, was raised in ascites from nearly all women with ovarian cancer [34], but it does not appear to be produced by tumor cells.

Further Applications of Tumor Markers

The antigens recognized by monoclonal antibodies are found on tumor cells and provide an opportunity to target antibodies to tumors in vivo, either for diagnosis or therapy. Diagnostic radioimmunolocalization studies have been performed with antibodies to CA125, TAG-72, and HMFG2 antigens and the monoclonal antibody 791T36 [35-39]. Tumors, both primary or recurrent, can be imaged preoperatively, but this technique is still in its infancy. However, radiolabeled B72.3 antibody (Oncoscint) has a product license and is the
antibody most commonly used in clinical practice. It is now possible to produce smaller molecularly engineered antibody fragments with high affinity for their antigen. Engineered molecules are likely to increase significantly the sensitivity and specificity of radioimmunodetection.

Radioimmunotargeting has also been applied to ovarian cancer. Preliminary results of intraperitoneal radiolabeled antibody therapy, particularly in the adjuvant setting, are encouraging [40, 41]. Whether these radiolabeled antibodies produce their effect as an adjuvant by radiation or alteration of the host’s immunity through the idiotypic network is unclear. There is now a randomized trial in the UK comparing intraperitoneal radiolabeled HMFG2 antibody to no treatment after negative second-look laparotomy.

**Nonepithelial Ovarian Cancer**

A detailed discussion of tumor markers in ovarian germ cell tumors is beyond the scope of this review and is outlined in Table 5. α-fetoprotein and human β chorionic gonadotrophin are probably the best known tumor markers in clinical practice and are invaluable in the diagnosis, treatment, and follow-up of ovarian germ cell tumors. Serum placental alkaline phosphatase and lactate dehydrogenase are also sometimes helpful as markers of dysgerminoma. Stromal tumors comprise approximately 10% of ovarian cancer. Traditionally, stromal tumors produce estradiol, and this has been used as a biochemical tumor marker. Granulosa cell tumors—a subgroup of stromal tumors, causing approximately 2% of ovarian malignancies—have been demonstrated to produce both estradiol and inhibin [42]. Approximately 30% of granulosa cell tumors and most extraovarian recurrences do not produce estradiol. Inhibin is a polypeptide hormone produced by the granulosa cells of the ovary and inhibits follicle-stimulating hormone secretion by the anterior pituitary gland. It is a glycoprotein consisting of two subunits—β and α. Measurement of inhibin has been restricted by a lack of sensitivity and cross-reactivity with the active dimeric form and inactive α subunits. A new radioimmunoassay has now been developed which recognizes both subunits of inhibin and is more sensitive. Serum levels of inhibin have been demonstrated to correlate closely with clinical disease and, like CA125, can predict relapse sometime before it becomes symptomatic [43].

**Conclusions**

Serological markers provide a means of monitoring tumor activity at many stages of the disease—diagnosis, therapy, and relapse. However, it is important that they are used appropriately and their significance is understood. Knowledge about raised levels of CA125 often raises questions as well as answers; we need to be able to make use of the information available. Early knowledge about relapse does not necessarily help outcome, as better therapies are needed. Progress in therapy is likely to come from a combination of better drugs and a greater understanding of the biology of the disease. Study of serological and tumor-related surface markers needs to continue. Markers for ovarian cancer, and, in particular, CA125, have led the way for epithelial tumors and provide a valuable model for further studies.

**References**