In Search of the Ideal Tumor Marker for Epithelial Ovarian Cancer: Serum Antigen CA-125 versus HE4, and Others; Current Status and Clinical Utility

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ABSTRACT

Epithelial ovarian cancer (EOC) is the most lethal gynecologic malignancy; it primarily affects postmenopausal women, and the average age of onset is between 55 and 65 years in the USA. The prevalence is one in 2,500 postmenopausal women.

We don’t have ideal strategies for prevention and detection, and 80% of cases are locally advanced disease. A variety of biomarkers have been developed to monitor growth. CA-125 (MUC 16) has provided a useful serum tumor marker for monitoring the efficacy of cytotoxic chemotherapy and the early detection of relapse during the follow-up of patients.

Twenty per cent of all cases have little or no expression of CA-125 and it is necessary to explore new markers for early disease or screening. Several algorithms have been developed that calculate the risk of ovarian cancer on serial CA-125 values and refer patients at highest risk for transvaginal sonography, but the strategy is limited.

More than 30 serum markers have been evaluated alone and in combination with CA-125 by different investigators. Some of the most promising include: HE4, mesothelin, M-CSF, osteopontin, kallikreins, and the soluble growth factor receptor.

In this review, we describe the actual status of CA-125, new strategies for early detection, and the utility of the new markers in this disease. (J CANCEROL. 2014;1:9-15)

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INTRODUCTION

Epithelial ovarian cancer (EOC) is the sixth most common gynecologic malignancy, with a crude incidence rate of 4.7 per 100,000 in women aged < 50 years that increases to 29.6 per 100,000 in those aged 50-64 years\(^1\). It is the most lethal gynecologic malignancy, primarily affects postmenopausal women, and the average age of onset is between 55 and 65 years. In 80% of cases it presents with locally advanced stage III-IV disease according to the criteria of the Federation of Gynecology and Obstetrics (FIGO), perhaps due to the anatomical location of the ovaries, which contributes to a lack of clear early symptoms. Still, the survival rate for stage I disease is > 90%, whereas in advanced stages it decreases to 25-30%\(^2\). It is therefore essential to have effective screening methods or support in detecting tumor markers to allow a more timely diagnosis, adequate monitoring of treatment and support in providing patient follow-up and monitoring.

Cancer antigen 125 (CA-125) is the established biomarker for detecting recurrence and monitoring therapeutic response. However, its use for diagnosis is limited as its sensitivity is < 50% in early stage disease and it has low specificity. Indeed, this glycoprotein, antigen 125, is widely distributed in cells of mesothelial origin in benign as well as malignant conditions other than EOC\(^3\).

Among the wide range of biomarkers recently proposed to aid in improving diagnosis of women with EOC, human epididymis protein 4 (HE4) is the most promising. This biomarker was proposed to improve diagnostic specificity of CA-125, while maintaining the same sensitivity, but preliminary studies suggest that it is superior to CA-125 in the early stages and in strains of tumors of low malignant potential\(^4\).

The purpose of this review is to analyze and describe the studies relating to currently available biomarkers for EOC and to clarify the clinical application of these new diagnostic tools.

CA-125

Ever since Bast, et al.\(^5\) identified a mucin-type molecule designated CA-125/MUC16 using murine monoclonal antibodies (OC125) in patients with ovarian serous cystadenocarcinoma, serum antigen CA-125 has become the primary tumor marker for epithelial ovarian cancer.

Molecule CA-125 is a high molecular weight glycoprotein that is composed of a small transmembrane domain and a large glycosylated extracellular domain, with 60 tandem repeats that contain amino acids that bind the OC125 and M11 antibodies, which are antibodies used for the detection of CA-125\(^6\).

The first immunoassay for CA-125 was marketed in 1983. This first generation of tests used the OC125 antibody for both capture and detection of CA-125. Subsequently, a second-generation assay (CA-125II) was developed using the M11 antibody to capture and the OC125 antibody as tracer because they both detect different epitopes.

At present there are several different immunoassays that have been adapted to automated platforms, but although the majority of manufacturers quote similar reference intervals, concentrations of CA-125 may vary due to differences in assay design and the agents used. For this reason, serial monitoring of CA-125 levels should be performed using the same immunoassay test and not interchanged with different methods\(^7\)\(^-\)\(^9\).

Normal levels of CA-125 were arbitrarily set at 35 U/ml. Bast reported that in a group of 888 patients, only 1% of healthy patients had CA-125 levels > 35 U/ml. Furthermore, elevated levels may also be found in 5% of those with benign diseases.
trials are underway that include use of CA-125 in a multimodal strategy together with transvaginal ultrasound, monitoring of sequential changes in serial CA-125 levels, and its use in combination with new tumor markers.

CA-125 is generally accepted more as an adjunctive tool for the differentiation between benign and malignant pelvic masses in contrast to its use in early detection, particularly in postmenopausal women, in whom different studies have reported sensitivities of 71-78% and specificities of 75-94%. In postmenopausal women, CA-125 levels > 95 U/ml have a 95% chance (PPV, positive predictive value) of being indicative of a malignant pelvic mass.

Predictive value of CA-125

Use of CA-125 is recommended during primary therapy as a potential prognostic indicator, both in the preoperative and postoperative periods. In the preoperative period, concentrations of CA-125 > 65 U/ml have been associated with worse survival. A limitation of most of these studies is the small numbers of patients and the results were not validated as independent prognostic factors.

Several studies have focused on the use of CA-125 levels as a predictor of surgical outcomes, with a probability of achieving optimal postoperative results in 73-82% of patients with preoperative CA-125 levels < 500 U/ml. Unfortunately, the probability of false positives ranges from 14 to 52%. In the postoperative period, several CA-125 values have been used as a prognostic factor for survival. These include:

(i) The half-life of CA-125, which is monitored at the start of chemotherapy and during the first three courses; (ii) absolute levels prior to chemotherapy and after the second course have been considered to be a favorable prognostic factor, especially if CA-125 levels are normalized; arbitrary cut-off concentrations such as > 70 U/ml or < 10 U/ml have
been used for prognostic purposes; and finally (iii) nadir concentration, with levels > 20 U/ml as the lowest value during the course of chemotherapy being associated with a poor prognosis\textsuperscript{15,16}, but because the best prognostic factor for survival has a 20% chance of being false, it cannot be recommended for individual patient management\textsuperscript{17,18}.

**Monitoring response to treatment and follow-up using CA-125**

Follow-up of this disease is difficult as 50% of patients do not have measurable disease as defined by World Health Organization standard criteria or by the Response Evaluation Criteria in Solid Tumors group\textsuperscript{19}.

At present there are different guidelines that propose CA-125 be used to monitor response to chemotherapy. Rustin, et al. originally defined response as a 50-75% decrease in CA-125 after therapy\textsuperscript{20,21}. Most international organizations use these criteria to evaluate response to new treatments\textsuperscript{22}. Generally speaking, 50% response means a 50% fall in the CA-125 levels that is sustained for 20 days in patients who initially had two elevated samples. The definition for progression has likewise been made on the basis of change in CA-125. Progression has been defined according to pretreatment CA-125 concentration and levels at the end of treatment. It is important to take into account certain factors of uncertainty with regard to use of serial CA-125\textsuperscript{23}. Among these are the analytical variation of the assay used and intra-individual biological variations. A significant change must exceed random fluctuations caused by analytical and biological variations (10%)\textsuperscript{24}.

**Monitoring for recurrence using CA-125**

With regard to monitoring for recurrence, Vergote, et al. proposed scheduling follow-up visits, which would include evaluation of CA-125 levels at two or three month intervals during the first years. However, its use in this setting is controversial because recurrent ovarian cancer is incurable. For this reason assessment should be made of the side effects, the reduction in quality of life against the real benefit of palliative therapy, while also keeping in mind that there is a small subgroup of patients that may benefit from secondary cytoreduction and for whom systemic therapy may prove more effective when administered early.

Several studies have proposed a diagnosis of recurrence can be made based solely on elevations in CA-125, such as CA-125 levels doubling from its nadir after treatment.

Definitions of recurrence based on doubling of CA-125 levels:

- Elevation > 70 U/ml if CA-125 after treatment is < 35 U/ml;
- Elevation to double the nadir level achieved with treatment if CA-125 was not normalized after treatment;
- Elevation to double the nadir level if CA-125 after treatment was < 35 U/ml.

Nevertheless, it is very important to note that a definitive diagnosis of recurrence must be accompanied either by the appearance of new lesions on imaging studies or pathologic confirmation, and although clinical recurrence may be preceded by elevations of CA-125 levels over a short interval of 3-4 months, it is not recommended to initiate cytotoxic therapy without first obtaining definite evidence by imaging and/or biopsy of the recurrence.

In light of the above, the Mexican Consensus Conference approved the use CA-125 levels for differential diagnosis of pelvic masses, especially as a prognostic factor for postmenopausal women for monitoring of treatment, and for detecting recurrence\textsuperscript{25} (Table 1).
Table 1. Recommendations for use of CA125 in pelvic masses

<table>
<thead>
<tr>
<th>Use</th>
<th>American College of Physicians</th>
<th>EGTM 2005</th>
<th>ESMO</th>
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NEW STRATEGIES FOR EARLY DETECTION

The PLCO and UKCTOCS screening trials. As it is considered acceptable to perform transvaginal ultrasound in cases of elevated CA-125 levels and because recommendations without a firm basis to the general population to have a CA-125 blood test and pelvic ultrasound are widespread, two large randomized studies were designed to evaluate this routine clinical practice. The PLCO (Prostate, Lung, Colorectal, and Ovarian) Cancer Screening Trial screened 78,216 healthy women and found that, in general, performing these two tests does not improve early detection since 75% of the cases identified by this method were in clinical stages III and IV26.

In the UKCTOCS study (United Kingdom Collaborative Trial of Ovarian Cancer Screening), 202,638 postmenopausal women aged 50-74 years and at average risk were randomly assigned to a group: annual pelvic exam, annual determination of CA-125 levels, and pelvic transvaginal ultrasound, called the MMS group, or to another group where only annual screening of CA-125 levels was performed and in cases where they were found to be high, indication was given for transvaginal ultrasound, called the USS group. Comparison of the ultrasound alone with multimodal screening showed higher specificity in the second group (99.8 vs. 98.2%) and higher positive predictive value (35.1 vs. 2.8%; p < 0.001). Full results from this study are awaited in 2014-2015; there have been some reports since 2012 that confirm the benefits that timely detection in the early stages of the disease has on improvement of overall survival.

Differences in the results are due mainly to monitoring being conducted primarily by gynecologic oncologists using the ROCA (Risk of Ovarian Cancer Algorithm), which does NOT use a single cutoff level to define elevated CA-125 but instead uses the patient’s own serial measurements27.

NEW BIOMARKERS FOR SCREENING, DIAGNOSIS, AND MONITORING OF EPITHELIAL OVARIAN CANCER

Given the limitations of CA-125, various efforts to identify a more suitable biomarker have been reported. These markers include leptin, prolactin, OPN, IGF-II, MIF, CEA, CA 15-3, CA72 and M-CSF, Apo A1, transferrin (TF), etc. They have been evaluated alone and in combination, but have not improved on CA-125. Nonetheless, HE4 has stood out.

Human epididymis secretory protein 4 (HE4) is a glycoprotein secreted by the WFDC2 gene. Studies
focused on demonstrating the potential of HE4 as a biomarker for EOV suggest that it is elevated in 50% of cases in which the CA-125 levels are not elevated, due either to early stage disease or histological type, leading the FDA to clear it for use as an additional screening option for EOC. The combined use of HE4 and CA-125 has been proposed in the differential diagnosis of pelvic masses and has attracted significant interest since Moore, et al. recognized the superiority of this panel of biomarkers in the diagnosis of pelvic masses, and their results have been corroborated by other groups.

ROMA AND THE OVA1 TEST

On the basis of the higher sensitivity and specificity resulting from the combination of these biomarkers, new diagnostic models have been developed. A new diagnostic test based on a scoring system called ROMA (Risk of Ovarian Malignancy Algorithm) has been developed by Skates, et al. that incorporates both CA-125 and HE4. In both premenopausal and postmenopausal women it provides sensitivity of 74% at specificity of 74.9% and sensitivity of 100% at specificity of 74%30. Because of these results, the FDA approved this test for the diagnosis of pelvic masses in both pre- and postmenopausal women, although not every group has been able to reproduce these results.

The FDA approved OVA1 on September 11, 2009. This test combines CA-125, TTR, ApoA, Beta-2 microglobulin, and TF, identified through serum proteomics using SELDI-TOF-MS, although no validation studies have demonstrated the superiority of OVA1 to CA-12531.

CONCLUSIONS

The search for better biomarkers to improve screening tools has led to the combination of biomarkers that achieve sensitivity and specificity greater than 90%. Nevertheless, these panels of biomarkers or algorithms have not been shown to be superior to what is currently provided by CA-125 alone with respect to feasibility and costs; we will need to continue to evaluate techniques, algorithms that will lead to improvements in our clinical practice. It is important now to know that a combination of CA-125 and HE4 improves diagnostic possibilities of pelvic masses. However, with regard to evaluation of treatment, prognosis, and follow-up, we do not yet have any biomarkers that are superior to CA-125.

REFERENCIAS