

## HE4 and CA72.4 are Useful Biomarkers in the Follow-up of Epithelial Ovarian Cancer

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### Abstract

The aim of this study was to investigate the role of serum biomarkers HE4 and CA72.4 at diagnosis and in the follow-up of patients with epithelial ovarian cancer (EOC). Seventy-eight patients with EOC were included and 40 of them were monitored during the follow-up. Serum levels of HE4 and CA72.4 were determined for all patients at diagnosis. Among these patients, the number of cases with an elevated level of each individual marker HE4 and CA72.4 was 85% and 72% respectively. A statistically significant difference was observed between the positivity of HE4 in comparison with Ca72.4 ( $p<0.02$ ). In the follow-up period, we observed that tumor marker levels showed fluctuations during chemotherapy. As we combined the individual biomarkers, we observed increased values in 75% of the patients for HE4 with CA72.4. In conclusion, our study has shown that the association of the biomarkers HE4 and CA72.4 provides a valuable contribution to the follow-up of EOC.

**Keywords:** Epithelial Ovarian Cancer; Biomarkers, HE4, CA72.4

### Introduction

Epithelial ovarian cancer (EOC) is the second most common form of gynecologic cancer and the first cause of death from gynecologic malignancy in the western hemisphere [1]. At present the global incidence is approximately 165,000 cases per year [2]. The diagnosis of ovarian cancer at early stage has an excellent prognosis if treated, but due to its biological characteristics and the lack of screening programs the disease diagnosis occurs in most cases (80%) when it is already in advanced stages (FIGO III-IV). Unfortunately, in these patients the median survival ranges are from 18 to 24 months with a probability of 80% of recurrence of the disease within five years [3-5].

For these reasons, numerous studies have been conducted in order to identify a marker with high specificity and sensitivity for the diagnosis of the ovarian cancer. Attention has been focused recently on a new biomarker, Human epididymis specific protein 4 (HE4), is commonly over-expressed in ovarian neoplastic tissue. Elevated HE4 protein levels have been found in serum of patients with early stage ovarian cancer, thus, it may be considered an early indicator of disease recurrence [6]. HE4 is not influenced by the hormonal fluctuations during the menstrual cycle [7].

Another EOC biomarker, CA72.4 antigen, a glycoprotein which increases in gastric, colon, breast and ovarian adenocarcinomas is not affected by pregnancy or the menstrual cycle levels only slightly rise with endometriosis, benign ovarian tumors, or inflammatory conditions [8-10].

In the clinical management of patients with EOC, the combination of multiple ovarian tumor markers is promising. Reliable tumor markers can be used to monitor response to treatment and detect early recurrence of disease, since serum elevations may precede clinical or radiological detectable disease by a median time of 2 to 6 months [6].

The aim of the present study was to evaluate whether the combination of serum biomarkers HE4 and CA72.4 can be used to monitor ovarian cancer and whether their combined use improves the management and follow-up of patients with EOC.

### Material and Methods

#### Patients

Serum samples were collected and analyzed from 78 subjects, attending the Gynecologic Oncology Unit of the Sapienza University of Rome, scheduled to undergo radical surgery. Clinical and histological diagnosis was defined according to the stadiation criteria of the International Federation of Gynecology and Obstetrics (FIGO) and grading was obtained according to the current classification and guidelines [22-23]. The mean age was  $65.32 \pm 11.73$  years (mean $\pm$ SEM), 88% (68/78) of the patients were post-menopausal.

The clinico-pathologic characteristics of the 78 patients with EOC are summarized in (Table 1). Twelve were diagnosed as FIGO stage I, 6 stage II, 6 stage III, and 54 stage IV.

All cancer patients were investigated for the determination of

Histology	Serous	Mucinous	Undifferentiated	Others
	53.8%	10.2%	28.2%	7.7%
Grade	1	2	3	
		43.6%	56.4%	
Stage	I	II	III	IV
	15.4%	7.7%	7.7%	69.2%

Table 1: Clinicopathological characteristics of the patients.

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serum levels of ovarian biomarkers HE4 and CA72.4 before surgery and 40 of the 78 patients were monitored during the follow-up period (4-6 months – 2 years). A written informed consent was obtained from all patients prior to collection of blood samples.

### Sample preparation

All sera were acquired following a standard collection protocol. Briefly, samples were collected in a Red Top Vacutainer, clotted 60-90 min and centrifuged for 10 min at 1300 x g. The serum fractions were aliquoted and stored at -80 C until analysis.

### HE4 assay

HE4 levels were determined using the HE4 EIA assay (Fujirebio Diagnostics). The HE4 EIA is a solid phase, non competitive immunoassay based upon the direct "sandwich" technique using two monoclonal antibodies, 2H5 and 3D8, directed against two epitopes in the C-WFDC domain of HE4. Controls or patient serum samples and standards were incubated with biotinylated anti-HE4 monoclonal antibody 2H5 aliquots in streptavidin coated microstrips. HE4 present in standards or serum samples was adsorbed to the streptavidin coated microstrips by the biotinylated anti-HE4 monoclonal antibody during the incubation period. The strips were then washed and incubated with HRP labeled anti-HE4 monoclonal antibody 3D8. After washing, buffered substrate/chromogen reagent was added to each well and the enzyme reaction was allowed to proceed. During the enzyme reaction a blue color developed if the antigen was present. Color intensity was directly proportional to the amount of HE4 present in the samples. Normal values of HE4 were considered to be less than 150 pmol/L, according to the manufacturer's indications.

### CA72-4 assay

CA72-4 was detected utilizing a solid phase two-site immunoradiometric ELSA- CA72-4 assay (Cisbio Bioassays, France). Two monoclonal antibodies were prepared against sterically remote antigenic sites on the TAG 72 molecule: the first was coated on the ELSA solid phase, the second, radiolabeled with iodine 125, was used as tracer. TAG 72 molecules present in the standards or the samples to be tested were "sandwiched" between the two antibodies. Following the formation of the coated antibody/antigen/ antibody sandwich, the unbound tracer was easily removed by a washing step. The radioactivity bound to the Elsa was proportional to the concentration of TAG 72 present in the sample. Normal levels of CA72-4 were considered to be less than 3 U/ml.

### Biomarker distribution in groups

The values above the reference limit of both markers determined in all patients were arbitrarily distributed in quartiles. Evaluating HE4, quartile I: values from 150 to 249 pmol/L; quartile II: values from 250 to 349 pmol/L; quartile III: values from 350 to 449 pmol/L; quartile IV: values from 450 to >850 pmol/L. Whereas CA72.4, quartile I: values from 3,1 to 10 U/ml; quartile II: values from 11 to 20 U/ml; quartile III: values from 21 to 40 U/ml; quartile IV: values from 41 to > 112 U/ml.

### Statistical analysis

The statistically significant difference were assessed using chi-square test for categoric variables (SPSS statistical software, version 13, Illinois, USA).

### Results

Serum levels of HE4 and CA72.4 were determined for all patients

at first diagnosis of ovarian epithelial cancer. Among these patients, the number of cases with an elevated level of each individual marker HE4 and CA72.4 was, 85% (66/78) and 72% (56/78) respectively.

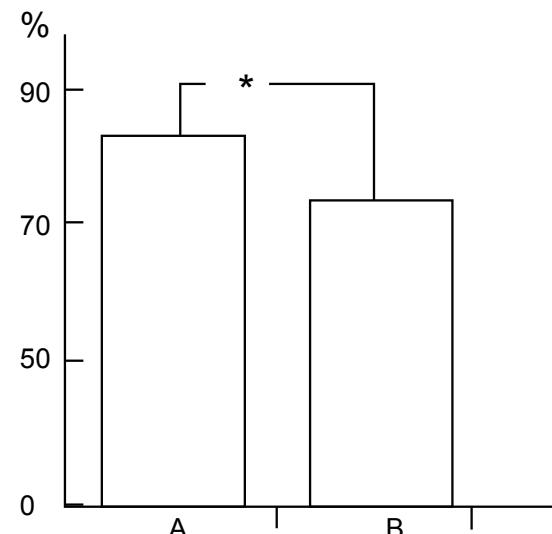
A statistically significant difference was observed between the positivity of HE4 in comparison with Ca72.4 ( $p<0.02$ ) (Figure 1).

The values of HE4 in 66 patients with EOC were distributed in the quartiles as follows: 20 in quartile I, 8 in quartile II, 6 in quartile III and 32 in quartile IV.

Whereas, the values of Ca72.4 in the 56 patients distributed in quartiles were the following: 30 quartile I, 6 quartile II, 2 quartile III and 18 quartile IV (Table 2). The combination of HE4 and Ca72.4 was 69% (27/39).

Forty of the 78 cancer patients enrolled were monitored for a period of 4-6 months to 2 years. 34 of the 40 patients (85%) observed during disease progression and relapse, confirmed clinically and with imaging techniques, had elevated levels of HE4 and CA72.4.

The HE4 levels were distributed in the following quartiles: 14 in quartile I, 8 in quartile II, 4 in quartile III and 8 in quartile IV, whereas the distribution of the CA72.4 quartiles were the following: 18 quartile I, 6 quartile II, 2 quartile III and 8 quartile IV (Table 3).



**Figure 1:** Percentages of HE4 and CA72.4 positive patients at first diagnosis of ovarian epithelial cancer. A, represents the percentage of patients with elevated biomarker HE4; B, represents the percentage of patients with elevated biomarker CA72.4. Statistically significant difference was observed between HE4 vs CA72.4 (\*  $p<0.02$ ).

	I quartile	II quartile	III quartile	IV quartile
HE4	20	8	6	32
CA72.4	30	6	2	18

**Table 2:** Serum HE4 and CA72.4 concentrations in all patients included in the study subdivided into quartiles.

	I quartile	II quartile	III quartile	IV quartile
HE4	14	8	4	8
CA72.4	18	6	2	8

**Table 3:** HE4 and CA72-4 concentrations in patients during the follow-up subdivided into quartiles.

Only 2 of the 40 patients analyzed during the follow-up period showed an increased level of CA 72.4 during relapse.

## Discussion

Epithelial Ovarian cancer (EOC) is the sixth most cancer in women in the European Union and represents the first cause of death of oncologic disease [1].

New and innovative approaches are currently used to better define the diagnosis and clinical management of patients affected by epithelial ovarian cancer.

The search for new ovarian cancer biomarkers has led to the identification of several molecules with a potential role for early diagnosis and triaging of adenexal mass.

Recent reports have demonstrated that diagnostic accuracy can be improved utilizing a new biomarker, HE4 (Human Epididymis Protein 4), whose expression closely correlates with EOC in women with advanced stage ovarian cancer [6, 11-14].

Serum tumor marker CA72.4 has also been shown to be elevated in a variety of neoplasias including EOC [8-10].

In the present study we confirmed the importance of HE4 since we have demonstrated that the marker is strongly expressed at diagnosis in high percentage of patients affected by EOC. [15-17].

Recently, we have demonstrated that the expression of HE4 during disease recurrence, precedes the rise of the expression of CA125 by a period of 6 months to 4 years. Therefore, HE4 may be considered a good biomarker for detecting early recurrence of disease as well as an ideal EOC marker for therapeutic strategies during relapse [6].

In this study, the expression HE4 and CA72.4 was evaluated prospectively in 40 patients during a follow-up period of about 2 years. An increased values of HE4 and CA72.4 has been observed in patients during the follow-up period. From a clinical point of view these increased levels correlated with disease recurrence confirmed with imaging. An important data which demonstrates the improved performance of CA72.4 during the follow-up period, is the observation that two of the 40 patients during relapse was positive only for CA72.4.

In conclusion this study demonstrated that the combination of HE4 with CA72.4 may have a predictive value in evaluating patients with disease recurrence of EOC.

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