

## Rapid Communication

# Preliminary Report of Routine use of CA-125 and HE-4 in 26 Consecutive Patients Operated in a National Cancer Center

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## Abstract

**Background:** The role of serological tumour markers for early diagnosis of ovarian cancer is still debated. Actually, Carbohydratic Antigen 125 (CA 125), is widely used in primary diagnosis of cancer but it has a particular role in monitoring response to treatment in ovarian cancer. Due to the low specificity, new strategies and new biomarkers are needed. Recently another glycoprotein, called human Hepididymis Protein 4 (HE4), has been proposed as a circulating marker for ovarian cancer. We evaluated the potential role of CA 125 and HE4 on a consecutive series of patients undergoing surgery at a National Cancer Center in order to evaluate if routine use of these markers may help the clinicians to establish the diagnosis and, thus, the correct treatment option.

**Methods:** Twenty six serum samples were obtained from peripheral blood of the same number of consecutive patients submitted to surgical treatment. HE4 and CA125 levels were detected and cross related to clinical-pathological characteristics of the patients enrolled. The results were analysed by one way ANOVA analyses and by t-test.

**Results:** There was no statistically significant difference between patients with any kind of cancer and patients with benign disease for HE-4 (p-value>0,05), while the difference between benign disease and cancer patients for CA125 was significant (p-value 0,0411). Among the study population, the highest values of CA 125 was observed in endometriosis patients as compared to cervical and vulvar cancer. Infact, among the 26 patients, 8 patients affected by benign disease had CA -125 (Average: 72, 81 pmol/ml) serum values higher than normal levels as compared to 7 patients with cervical cancer (Average: 19,83 pmol/ml) and 9 patients affected by vulvar cancer (Average: 15,01 pmol/ml). In reverse, patients with benign disease experienced, on average, lower values of HE-4 as compared to cervical and vulvar cancer. In fact, the 8 patients affected by benign disease had HE serum level's average equal to 66,8 pmol/ml while those affected by cervical and vulvar cancer had HE serum level's average respectively of 76,67 and 99,5 pmol/ml. Both CA125 and HE4 expression resulted statistically significant comparing pre-menopausal and post-menopausal values.

**Conclusion:** In our experience, CA 125 seems to be more suitable if compared to HE4, but, now studies on larger cohort are in progress with the aim to establish the potential of HE4.

## Introduction

Ovarian cancer is the most lethal gynaecological neoplasm and is the fifth leading cause of cancer death in women. This neoplasm presents with an specific symptomatology and is usually diagnosed in a late phase, when the chances of recovery are compromised [1].

The role of serological tumour markers for early diagnosis of cancer is still debated. Carbohydratic Antigen 125 (CA 125) is a glycoprotein produced from the uterus, the fallopian tubes and from the cells that line the organs of the respiratory tract and the abdomen. It is widely used in primary diagnosis of cancer but it has a particular role in monitoring response to treatment in ovarian cancer. However, CA125 has a low specificity, as high levels of may be observed also

in pregnancy, endometriosis, and/or other malignancy such as lung, breast, and colon [2].

More recently another glycoprotein, called human Hepididymis Protein 4 (HE4), has been proposed as a circulating marker for ovarian cancer. Codified by WDFDC2 gene, HE4 was initially identified in hepididymis tissue; subsequently it was found expressed not only in ovarian cancer but also in several normal tissues such as reproductive and lung epithelium. From the first article, in which Hellstrom et al. [3] reported the potential role of HE4 in distinguishing patients with malignant ovarian disease from those with benign ovarian disease with higher specificity than CA125, several authors have investigated this protein in serum for diagnostic and prognostic purposes [4].

The aim of this study was to evaluate the potential role of CA 125 and HE4 on a consecutive series of patients undergoing surgery at a National Cancer Center in order to evaluate if routine use of these markers may help the clinicians to establish the correct treatment option.

## Materials and Methods

Serum samples were obtained from peripheral blood of 26 consecutive patients submitted to surgical treatment at the Gynecologic Oncology Unit, National Cancer Center, "Giovanni Paolo II", Bari, Italy, the day before surgery. Written informed consent was obtained from each patient and the protocol was approved by the Institutional Review Board. In order to minimize variable effects due to sample collection, processing and storage temperature, all blood samples were managed in the same manner without any protocol amendment during the entire collection period. The blood specimens were collected in serum separation tubes (Becton Dickinson, Franklin Lakes, NJ, USA), allowed to clot for thirty minutes, and then centrifuged at 3500 rpm for 15 minutes for the subsequent routinely use.

HE4 and CA125 levels were detected with an immunoenzymatic assay (FUJIREBIO Inc., Japan and Roche Diagnostics S.p.A., respectively) according to manufacturer's guidelines.

Clinico-pathologic characteristics of the patients enrolled in the study were collected.

The results were analyzed by one-way ANOVA analyses for evaluation of the marker's levels in the various pathologies and t-test for comparison between menopausal status.

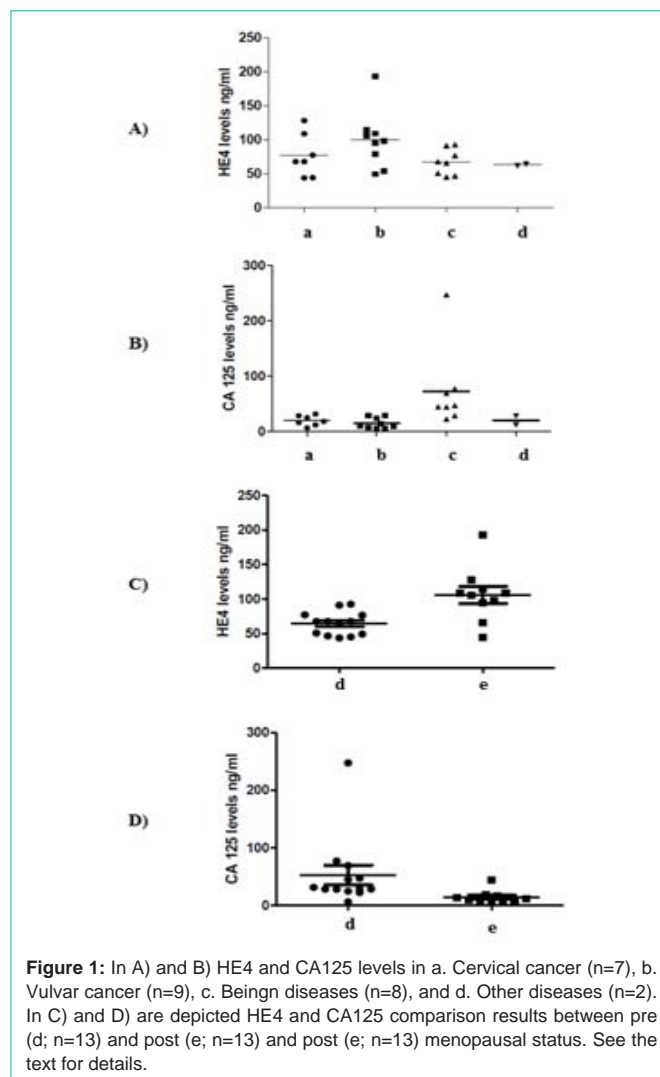
## Results

Median age of the 26 patients was 60 years (range 42 -83 years). Half of them (n=13) were in pre-menopausal phase. Seven patients had cervical cancer, nine had vulvar cancer (two Paget disease), one each had endometrial stroma sarcoma and trophoblastic disease, and eight patients were found to have benign disease at final pathologic examination benign (endometriosis, myomas).

Median values of serum Ca125 and HE4 levels are depicted in Table 1. There was no statistically significant difference between patients with any kind of cancer and patients with benign disease for HE-4 while the difference between benign disease and cancer patients for CA125 was significant. Among the study population, the highest value of CA 125 was observed in endometriosis patients as compared to cervical and vulvar cancer. In fact 8 patients affected by benign disease had CA -125 (Average: 72,81pmol/ml) serum values higher than normal levels as compared to 7 patients with cervical cancer (Average: 19,83pmol/ml) and 9 patients affected by vulvar cancer (Average: 15,01 pmol/ml) see Table 1 for details.

In reverse, patients with benign disease experienced, on average, lower values of HE4 as compared to cervical and vulvar cancer. In fact, the 8 patients affected by benign disease had HE serum level's average equal to 66,8 pmol/ml while those affected by cervical and vulvar cancer had HE serum level's average respectively of 76,67 and 99,5 pmol/ml.

Analyzing data with respect to menopausal status both CA125



and HE-4 expression resulted statistically significant comparing pre-menopausal and post-menopausal values see Table 1 for details.

## Discussion

Ovarian cancer represents the first among the neoplasms of the reproductive system in women and it is in fourth place among the causes of death by cancer in women all over the world. Moreover, it is one of the most difficult oncological pathologies because it is often discovered late when the chances of recovery are compromised.

Early diagnosis is an essential element to reduce the poor prognosis and improve the quality of life of the patient: the diagnosis performed in the first or second stage of tumor development has a 5-year survival of more than 85% of cases, while at III and IV stage is less than 15%.

HE4 (Human Epididymis Protein 4) is a glycoprotein initially identified in the epididymis but normally expressed in the epithelial cells of the upper respiratory tract, in the pancreas and in the reproductive system. Recent studies have shown that HE4 is over-expressed in ovarian cancer, making it, together with CA125, a serological marker in the risk assessment of malignancy. Reviewing

**Table 1:** ANOVA analysis highlighted that the levels of HE4 in the 4 study groups are not statistically significant with respect to CA125. On the other hand both HE4 and CA125 were differentially expressed when compared pre and post-menopausal status.

**A) HE4 level's average in the 4 study groups.**

	HE4 in cervical cancer (n=7)	HE 4 in vulvar cancer (n=9)	HE 4 benign disease (n=8)	HE 4 in others (n=2)	P-value
Average (min-max)	76,67ng/ml (43–128)	99,5ng/ml (49,2–192,9)	66,98ng/ml (45–92,6)	62,9ng/ml (61,7-64)	0,1829

**HE4 level's average in menopausal status.**

	Pre-menopause (n=13)	Post-Menopause (n=13)	P-value
Average (Min-max)	64,56923ng/ml (43,7–92,6)	106,05ng/ml (44–192,9)	0,0048

**B) CA125 level's average in the 4 study groups.**

	CA125 in cervical cancer (n=7)	CA125 in vulvar cancer (n=9)	CA125 in benign disease (n=8)	CA125 in others (n=2)	P-value
Average (Min – max)	19,83ng/ml (6,5–31,7)	15,01ng/ml (6,4–29,4)	72,81ng/ml (22,7–247,3)	20,2ng/ml (12–28,4)	0,0411

**CA125 level's average in menopausal status.**

Pre-menopause	Post-Menopause	P-value
52,82308ng/ml (6,-247)	14,47ng/ml (6,4–44,6)	0,0026

past literature, HE4 studies exhibit a variable sensitivity and specificity; Park et al. aimed to compare the characteristics of HE4 and CA125 in various gynecologic and non-gynecologic diseases, and to evaluate the diagnostic performance of both CA125 and HE4 in discriminating ovarian cancer from other benign gynecologic diseases. They found a sensitivity of HE4 for detecting ovarian cancer was 44.8% (with respect to that of CA 125 55,2%) at 95% specificity [5], while Granato et al. found a sensitivity of 96% with a specificity of 44% [6].

In the middle, several authors, found good sensitivity and specificity, and moreover among these, Jacob et al. although the good sensitivity and specificity [7], concludes that HE4 do not adds any benefit in early detection of ovarian cancer.

On the other hand, HE4 is extremely useful in discriminating between ovarian cancer, cysts or benign ovarian masses and endometrial carcinoma. In our study we have tested both CA 125 and He4 in a consecutive and pre-surgery series of patients observed. According to Table 1 and Figure 1, we found that the two markers had a similar behavior with a slightly better sensitivity to HE4 in cervical and vulvar tumors. Furthermore, both HE4 and CA125 appeared to be differentially expressed in a statistically significant manner when compared to the menopausal status.

The results of our study suggest that CA 125 and HE4 should be included in the preliminary management of patients referred for surgical treatment in a Gynecologic Oncology Unit. A larger sample size is necessary in order to understand the role of these markers, particularly sensitivity and specificity. Probably their use might be more appropriate during follow-up of the disease.

## Conclusion

In our experience, CA 125 and HE4 seem to correlate in particular with the menopausal state and therefore with the age of the patients. On the contrary it does not seem that these markers correlate with the type of lesion. Considering the small number of patients, and therefore the limit of our results, we are continuing to enroll other patients to test our hypothesis on a larger population.

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