Journal of Clinical Laboratory Analysis 23 : 331–335 (2009)

The Utility of Serum Human Epididymis Protein 4 (HE4) in Patients With a Pelvic Mass

Martina Montagnana,[1]* Giuseppe Lippi,[1] Orazio Ruzzenente,[1] Valentina Bresciani,[2] Elisa Danese,[1] Silvia Scevarolli,[2] Gian Luca Salvagno,[1] Silvia Giudici,[2] Massimo Franchi,[2] and Gian Cesare Guidi[1]

[1]*Sezione di Chimica Clinica, Dipartimento di Scienze Morfologico-Biomediche, Università degli Studi di Verona, Verona, Italy*

[2]*Unità di Ostetricia, Dipartimento Materno ed Infantile, Ospedale Policlinico GB Rossi, Verona, Italy*

Aim: Although CA125 is the most widely used cancer marker in the diagnostic approach of pelvic masses in women, its clinical usefulness is limited because it lacks expression of the antigen in the early stages of disease. The human epididymis protein 4 (HE4) is frequently over-expressed in ovarian cancer, whereas its expression in normal tissues, including the ovary, is low. The aim of this study was to assess the concentration of both HE4 and CA125 in patients with different forms of benign and malign pelvic masses.

Methods: The study population included 99 patients with gynecological cancer (46 ovarian, 39 endometrial, 14 cervical) and 40 affected by benign disease (22 endometriosis and 18 benign ovarian mass). Twelve control subjects were also included in the study. In all the patients, serum samples were collected on the day before scheduled surgery.

Results: The median CA125 and HE4 serum levels were significantly higher among ovarian cancer patients as compared with healthy subjects and with those with benign mass, cervical, and endometrial tumors. The receiver operating characteristics curve analysis on healthy controls and patients with ovarian cancers revealed that HE4 had a significantly higher area under the curve when compared with CA125 (0.99 vs. 0.91), with a sensibility and specificity of 98 and 100%, respectively.

Conclusions: HE4 seems to be a promising ovarian cancer marker, and its measurement might improve the diagnostic approach to patients with pelvic masses. J. Clin. Lab. Anal. 23:331–335, 2009.

Key words: HE4; CA125; pelvic mass; ovarian cancer; cancer markers

INTRODUCTION

Ovarian cancer accounts for nearly 4% of all cancers among women (1), and it is the fifth more common cause of cancer death in women (2). Mortality is strongly related to disease stage: the 5-year survival is higher than 70% in stadium I or II, but decreases to 40 and 20% in stage III or IV, respectively (3). Given the high mortality rate of patients diagnosed with advanced cancers, the goal of gynecologists is to make a timely diagnosis and establish an early surgical and/or chemotherapic treatment (4). The common symptoms of ovarian cancer are vague and similar to those observed in other benign conditions (5). Therefore, a diagnostic approach based on the use of laboratory serum markers (mostly CA125) in association with ecocardiographic techniques is commonplace in these patients. However, this approach has several drawbacks, due to the low sensibility and relatively low specificity of CA125 (6).

The whey-acidic protein human epididymis protein 4 (HE4) gene is frequently over-expressed in ovarian cancers (7), suggesting that the promoter of the gene is highly transcriptionally active (8). Moreover, several studies have analyzed HE4 protein expression in ovarian

*Correspondence to: Martina Montagnana, Sezione di Chimica Clinica, Dipartimento di Scienze Morfologico-Biomediche, Ospedale Policlinico G.B. Rossi, Piazzale Scuro, 10, 37134—Verona, Italy. E-mail: martina.montagnana@med.lu.se

Received 22 April 2009; Accepted 15 July 2009
DOI 10.1002/jcla.20340
Published online in Wiley InterScience (www.interscience.wiley.com).

cancers, showing a higher protein expression in serous an endometrioid hystotype (9). Recent studies have also shown elevated HE4 protein levels in sera from patients with ovarian tumors, concluding that HE4 has a similar sensitivity to CA125, but an increased specificity in patients with malignancies as compared with those carrying a benign disease (10,11). Regardless of its overexpression in ovarian carcinoma, tissue expression of HE4 has been reported to be increased in some pulmonary, endometrial, and breast adenocarcinomas, mesotheliomas, and less often, gastrointestinal, renal, and transitional cell carcinomas (12). However, the serum levels of HE4 were not evaluated in these patients. Therefore, the aim of this study was to assess the tumor marker levels of both HE4 and CA125 in patients with different forms of benign and malign pelvic mass.

MATERIALS AND METHODS

Patient Population

Women diagnosed with a pelvic mass who were scheduled to undergo radical surgery from October 2007 to July 2008 were eligible for enrolment. The diseases were diagnosed perioperatively in laparoscopy or laparotomy, and confirmed by histopathological evaluation.

Patients affected by ovary cancer had the following histological subtypes (13): serous (30, 65.2%), endometrioid (6, 13.0%), clear cell (1, 2.2%), mucinous (5, 10.9%), germ cells (1, 2.2%), and ovary metastasis from gastrointestinal cancer (3, 6.5%). According to the stadiation criteria of the International Federation of Gynecology and Obstetrics (FIGO) (14), 10 patients were in stage I (21.7%), 2 in stage II (4.3%), 25 in stage III (54.3%), and 6 (13.0%) in stage IV.

According to World Health Organization/International Society of Gynecologic Pathologists (WHO/ISGP) classification (15), patients affected by endometrial cancer had the following histological subtypes: 36 (92.3%) endometrioid, 1 (2.6%) serous, and 2 (5.1%) squamous carcinoma. Thirty-two patients (82.1%) were in stage I, three (7.7%) in stage II, two (5.1%) in stage III and two (5.1%) in stage IV. Patients with benign ovarian mass displayed mucinous cystadenoma, serous cystadenoma, cystic teratoma, or ovarian simple cyst. Twelve out of the 22 patients with endometriosis displayed ovarian endometrioma.

Controls subjects were recruited among healthy hospital personnel undergoing the routine clinical and biochemical check-up. Each patient and control gave an explicit and written informed consent for participating in this study, which has been approved by our departmental ethical committee.

Laboratory Methods

Blood samples were collected in the morning before surgery on patients who had fasted overnight and rested for 20 min. Blood was drawn in vacuum tubes containing no additives (Becton-Dickinson, Oxford, UK). After centrifugation at 1,500*g* for 10 min at room temperature, serum was separated, stored in aliquots and kept frozen at -80°C until measurement.

Serum levels of CA125 were determined using a chemiluminescent enzyme immunoassay on the Liaison (DiaSorin, Saluggia, Italy). Intra and interassay coefficient of variation (CV) for this method are comprised between 1.4–2.2 and 4.6–5.8%, respectively.

Serum levels of HE4 were determined using ELISA kit developed by Fujirebio Diagnostic, Inc. (Malvern, PA) and were performed according to the manufacturer's specifications. The HE4 EIA is a solid-phase, noncompetitive immunoassay based on the direct sandwich technique using two mouse monoclonal antibodies, 2H5 and 3D8, directed against two epitopes in the C-WFDC domain of HE4. The total CV quoted by the manufacturer is $<10\%$.

Statistical Analysis

Values were finally reported as median (range). All the data were analyzed with SPSS statistical software (version 12.0; SPSS Inc. Chicago, IL). Tumor marker levels between groups were compared using the Kruskall–Wallis and Mann–Whitney test. The Spearman coefficient (r_s) was calculated to quantify correlation between variables. The level of statistical significance was set at $P<0.05$.

Receiver operator characteristic (ROC) curves were assessed for both HE4 and CA125. As no definitive diagnostic thresholds for these tests have been reported so far (intended as the cutoff point that separates normal subjects from cancer subjects), these were identified as the values showing the best diagnostic performance according to the ROC curve.

RESULTS

Results of HE4 measurements in different groups are shown in Table 1. The median CA125 and HE4 serum levels were significantly higher among all the ovarian cancer as compared with the sub-group of healthy subjects (CA125: 497.3 vs. 10.1 U/ml, $P<0.001$; HE4: 166.0 vs. 17.3 pmol/l, $P<0.001$) and patients with ovary benign disease (CA125: 497.3 vs. 18.6 U/ml, $P<0.001$; HE4: 166.0 vs. 39.4 pmol/l, $P<0.001$). Remarkably, CA125 and HE4 values were also statistically higher in patients with ovary cancer as compared with those suffering from endometrial tumor (CA125: 497.3 vs.

TABLE 1. HE4 and CA125 Levels in Different Groups

	Controls subjects ($n = 12$)	Ovarian benign masses ($n = 18$)	Endometriosis ($n = 22$)	Ovarian cancer ($n = 46$)	Endometrial cancer ($n = 39$)	Cervical cancer ($n = 14$)	P-value (K-W)
HE4, pmol/l	17.3 (8.8–29.4)$^{\#}$	39.4 (21.4–144.4)$^{*\#}$	47.4 (20.0–98.0)$^{*\#}$	166.0 (28.9–7437.3)*	72.2 (7.3–781.8)$^{*\#}$	88.0 (14.8–985.5)$^{*\#}$	<0.0001
CA125, U/ml	10.1 (4.3–34.7)$^{\#}$	18.6 (4.9–114.8)$^{\#}$	52.3 (14.9–90.9)$^{*\#}$	497.3 (6.4–7017.0)*	13.5 (6.1–1333.0)$^{\#}$	18.8 (6.7–95.7)$^{\#}$	<0.0001

HE4 and CA125 values were reported as median (range). P-values are evaluated by Kruskall–Wallis test. P-values between groups are evaluated by Mann–Whitney U test. $^{*}P<0.05$ vs. controls; $^{\#}P<0.05$ vs. ovary cancer.

13.5 U/ml, $P<0.001$; HE4: 166.0 vs. 72.2 pmol/l, $P=0.004$) and cervical tumor (CA125: 497.3 vs. 18.8 U/ml, $P<0.001$; HE4: 166.0 vs. 88.0 pmol/l, $P=0.028$). HE4 and CA125 levels in different FIGO stages of ovary cancer respect to controls are shown in Figure 1. A positive correlation between CA125 and HE4 levels ($r=0.74$, $P<0.001$) was observed in patients with ovarian cancer ($n=46$).

The ROC curve analysis of the diagnostic performance on healthy controls and patients with ovarian cancers revealed that HE4 had a significantly higher area under the curve (AUC) when compared with CA125 (Fig. 2). The sensibility and the specificity of CA 125 using a cut-off level of 37 U/ml were 83 and 100%, respectively (AUC = 0.91, $P<0.001$), whereas the sensitivity and the specificity of HE4 using a cut-off level of 30 pmol/l were 98 and 100%, respectively (AUC = 0.99, $P<0.001$). The analysis of the diagnostic performances on patients with ovarian cancers and with benign ovary mass do not show a better performance of HE4 when compared with CA125 (AUC = 0.87 vs. 0.88).

DISCUSSION

Despite the relatively low prevalence (30–50 cases/100,000 women), ovarian cancer still represents the fifth most common cause of cancer death in women (2). CA125 (MUC16) is useful for differentiating benign from malignant pelvic masses and it can be used to assess response to treatment, but is not sensitive or specific enough to justify a population screening (16–18). The major drawbacks of using CA125 as an initial step in such a screening strategy is that up to 20% of ovarian cancers lack expression of the antigen (19). Accordingly, other serum tumor markers that can be detected in ovarian cancers, especially those lacking CA125 expression, might improve the sensitivity for early detection (20).

HE4, a WAP four disulfide core domain protein, has been recently identified as the candidate molecular marker for ovary cancer. It has been previously established that HE4 is distributed in a region of the cytoplasm with a perinuclear pattern reminiscent of the endoplasmic reticulum and the Golgi apparatus (9). In ovarian carcinoma cells, but not in normal tissues, the HE4 gene product is *N*-glycosylated and secreted into the extracellular environment. Therefore, glycosylated HE4, with an apparent molecular weight of 25 kDa, may be secreted and then detectable in the bloodstream of patients with ovarian carcinoma (9).

In agreement with recent publications (11,21), we observed that the release of HE4 appears to be earlier than that of CA125, and HE4 levels are significantly higher in early stages (FIGO I–II) of patients with ovary

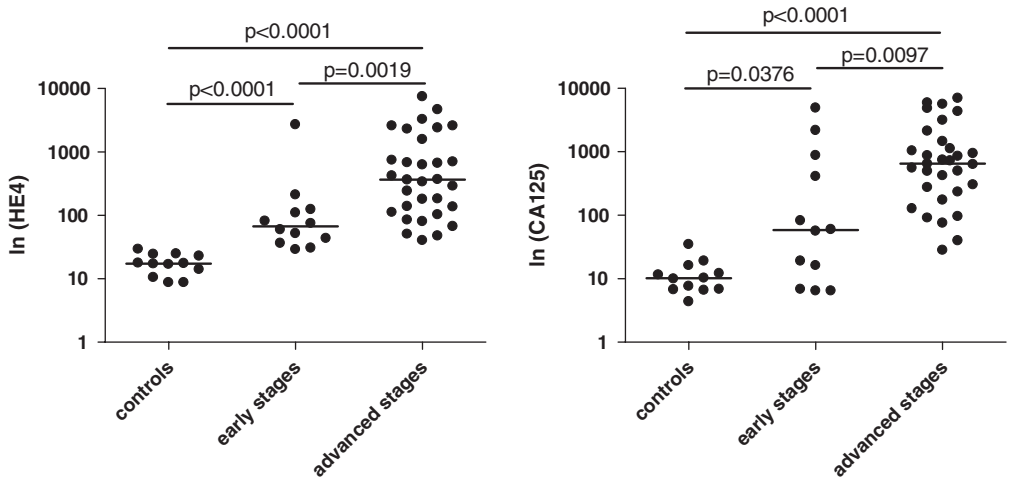

Fig. 1. Serum HE4 (pM) and CA125 (U/ml) concentrations as ln-transformation in patients with ovarian cancer in different FIGO stages of ovarian cancer respect to controls. Early stages, stages I and II; advanced stages, stages III and IV. *P* values between groups are evaluated by Mann–Whitney *U* test.

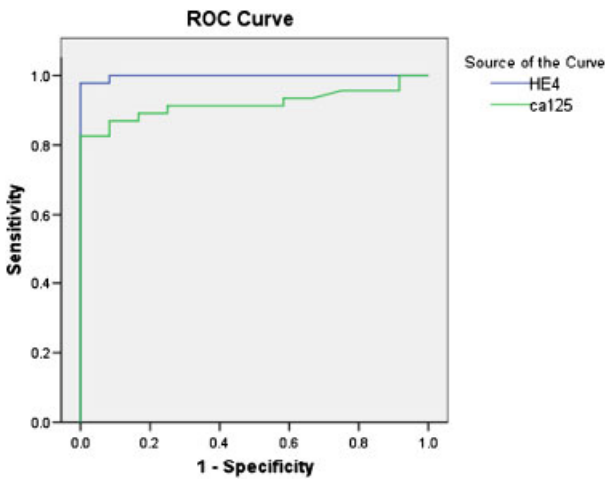

Fig. 2. Analysis of the diagnostic performances (ROC curves) on healthy controls and patients with ovarian cancer (AUC = 0.99 for HE4, AUC = 0.91 for CA125; $P < 0.0001$).

cancer as compared with control subjects. In line with this finding, CA125 levels are not increased in nearly 40–50% of early stage ovary cancer (20), and the diagnostic sensitivity of HE4 in early stages is much higher that that of CA125 (82.7% vs. 45.9%) (11). Different biochemical explanations can be hypothesized. First, CA125 levels seem to be more related to cell growth than HE4 (22). Moreover, HE4 is a high molecular weight glycosylphosphoinositol-linked glycoprotein (about 80 kDa), different from the secreted form, which is characterized by an apparent molecular weight of 25 kDa (9,23). It is hence reasonable that the low molecular weight could explain the early release of this form. Then, the mechanism of CA125 secretion from ovarian tumors seems to be more complicated than that of HE4. In fact, CA 125 undergoes two possible events: proteolytic remodeling in the C-terminal SEA domain with cleavage of the luminal N-terminal domain (24,25), or alternatively spliced mRNAs are generated that lack the TM region (23).

The main limitation of HE4 might be the relative low specificity. Although the serum measurement of HE4 may have an advantage over CA125, because the former is less frequently raised in patients with nonmalignant ovary disease (10), we observed that the level of HE4 might also be modestly increased in patients with benign ovary diseases. However, the magnitude of increase was ten times lower than that observed in patients with ovarian cancers (Table 1). Patients affected by endometrial and cervical carcinoma had also higher levels of HE4 as compared with the control population, but these values were still significantly lower than those observed in patients affected by ovary cancer (Table 1). In agreement to previous observations (26), HE4 but not CA125 levels are also increased in endometrial cancer patients, suggesting an additional clinical use for this marker (26).

Taken together, the results of this investigation attest that HE4 might be a promising marker for early differentiation of pelvic masses, showing diagnostic performances globally better than those of CA125, which is the conventional marker in the workout of ovarian cancers. Indeed, large clinical studies are necessary to support these findings, to confirm our diagnostic thresholds and to assess the potential usefulness of HE4 testing in the screening of high-risk populations.

REFERENCES

1. Colombo N, Van Gorp T, Parma G, et al. Ovarian cancer. Crit Rev Oncol Hematol 2006;60:159–179.

2. Pal T, Permuth-Wey J, Sellers TA. A review of the clinical relevance of mismatch-repair deficiency in ovarian cancer. Cancer 2008;113:733–742.
3. Heitz APM, Odicino F, Maisonneuve P, et al. Carcinoma of the ovary. Int J Gynecol Obstet 2006;95:S161–S192.
4. Dunleavey R. Importance of early diagnosis in managing ovarian cancer. Nurs Times 2006;102:28–29.
5. Evans J, Ziebland S, McPherson A. Minimizing delays in ovarian cancer diagnosis: An expansion of Andersen's model of "total patient delay". Fam Pract 2007;24:48–55.
6. Schwartz DR, Wu R, Kardia SL, et al. Novel candidate targets of beta-catenin/T-cell factor signaling identified by gene expression profiling of ovarian endometrioid adenocarcinomas. Cancer Res 2003;63:2913–2922.
7. Grisaru D, Hauspy J, Prasad M, et al. Microarray expression identification of differentially expressed genes in serous epithelial ovarian cancer compared with bulk normal ovarian tissue and ovarian surface scrapings. Oncol Rep 2007;18:1347–1356.
8. Berry NB, Cho YM, Harrington MA, Williams SD, Foley J, Nephew KP. Transcriptional targeting in ovarian cancer cells using the human epididymis protein 4 promoter. Gynecol Oncol 2004;92:896–904.
9. Drapkin R, von Horsten HH, Lin Y, et al. Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. Cancer Res 2005;65:2162–2169.
10. Hellström I, Raycraft J, Hayden-Ledbetter M, et al. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. Cancer Res 2003;63:3695–3700.
11. Havrilesky LJ, Whitehead CM, Rubatt JM, et al. Evaluation of biomarker panels for early stage ovarian cancer detection and monitoring for disease recurrence. Gynecol Oncol 2008;110:374–382.
12. Galgano MT, Hampton GM, Frierson Jr HF. Comprehensive analysis of HE4 expression in normal and malignant human tissues. Mod Pathol 2006;19:847–853.
13. Heintz AP, Odicino F, Maisonneuve P, et al. Carcinoma of the ovary. FIGO 6th Annual Report on the Results of Treatment in Gynecological Cancer. Int J Gynaecol Obstet 2006;95:S161–S192.
14. Shepherd JH. Revised FIGO staging for gynaecological cancer. Br J Obstet Gynaecol 1989;96:889–892.
15. Amant F, Moerman P, Neven P, Timmerman D, Van Limbergen E, Vergote I. Endometrial cancer. Lancet 2005;366:491–505.
16. Bast Jr RC, Feeney M, Lazarus H, Nadler LM, Colvin RB, Knapp RC. Reactivity of a monoclonal antibody with human ovarian carcinoma. J Clin Invest 1981;68:1331–1337.
17. Rustin GJ, Bast Jr RC, Kelloff GJ, et al. Use of CA-125 in clinical trial evaluation of new therapeutic drugs for ovarian cancer. Clin Cancer Res 2004;10:3919–3926.
18. Jacobs I, Oram D, Fairbanks J, Turner J, Frost C, Grudzinskas JG. A risk of malignancy index incorporating CA 125, ultrasound and menopausal status for the accurate preoperative diagnosis of ovarian cancer. Br J Obstet Gynaecol 1990;97:922–929.
19. Bast Jr RC, Brewer M, Zou C, et al. Prevention and early detection of ovarian cancer: Mission impossible? Recent Results Cancer Res 2007;174:91–100.
20. Rosen DG, Wang L, Atkinson JN, et al. Potential markers that complement expression of CA125 in epithelial ovarian cancer. Gynecol Oncol 2005;99:267–277.
21. Moore RG, Brown AK, Miller MG, et al. The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. Gynecol Oncol 2008;108:402–408.
22. Ayhan A, Guven S, Guven ES, Kucukali T. Is there a correlation between tumor marker panel and tumor size and histopathology in well staged patients with borderline ovarian tumors? Acta Obstet Gynecol Scand 2007;86:484–490.
23. Yin BW, Lloyd KO. Molecular cloning of the CA125 ovarian cancer antigen: Identification as a new mucin, MUC16. J Biol Chem 2001;276:27371–27375.
24. Ligtenberg MJ, Kruijshaar L, Buijs F, van Meijer M, Litvinov SV, Hilkens J. J Biol Chem 1992;267:6171–6177.
25. Boshell M, Lalani E-N, Pemberton L, Burchell J, Gendler S, Taylor-Papadimitriou J. The product of the human MUC1 gene when secreted by mouse cells transfected with the full length. cDNA lacks the cytoplasmic tail. Biochem Biophys Res Commun 1992;185:1–8.
26. Moore RG, Brown AK, Miller MG, et al. Utility of a novel serum tumor biomarker HE4 in patients with endometrioid adenocarcinoma of the uterus. Gynecol Oncol 2008;110:196–201.