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BENEFITS HUMAN EPIDYDIMIS PROTEIN (HE4) COMPARED TO TRADITIONAL USED TUMOR MARKERS IN GYNECOLOGICAL ONCOLOGY

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Summary

Ovarian cancer is, with its high incidence and mortality, a worldwide problem. One reason for this is the lack of symptoms. The second reason is practically non-existant screening for ovarian cancer. Until recently, the only routinely used marker for ovarian abnormalities was CA125. Determination of HE4 levels, together with those of CA125 and the calculation of the ROMA index, is a suitable method for improving primary detection of ovarian cancer. The measurement of serum HE4 is a useful method for differential diagnosis between benign gynecologic disease and ovarian cancer.

KEY WORDS: *ovarian cancer, CA125, HE4 (Human epidydimis protein), ROMA (Risk of Ovarian Malignancy Algorithm) index.*

PREDNOSTI HUMANOG EPIDIDIMALOG PROTEINA (HE4) U ODNOSU NA TRADICIONALNO KORIŠTENE TUMORSKE MARKERE U GINEKOLOŠKOJ ONKOLOGIJI

Sažetak

Rak jajnika je globalni javnozdravstveni problem radi visoke učestalosti i smrtnosti. Jedan od razloga za to je nedostatak jasnih kliničkih simptoma bolesti. Drugi razlog je praktički nepostojanje metode probira za rak jajnika. Donedavno, jedini rutinski koristan tumorski marker za karcinom jajnika bio je CA125. Određivanje razine HE4, zajedno s CA125 i izračun indeksa ROMA pogodna je metoda poboljšanja dijagnostike primarnog otkrivanja raka jajnika. Mjerenje serumskog HE4 korisno je u razlikovanju benignih ginekoloških bolesti od karcinoma jajnika.

Ključne riječi: *rak jajnika, CEA 125, HE4 (Humani epidimelni protein), ROMA index*

INTRODUCTION

Despite the relatively low prevalence, ovarian cancer is the six leadingcause of death from cancer among women in Croatia. Distribution of newcancer cases in 2013 by site was 446/9769 ovarian cancer in females (5%). Incidence rate ovarian cancer is 20.1 and it is too high compared to the standardized rate of the world population, which is 10.8. (1). The most reliable, but not always easy approach to diagnose ovarian cancer relies on multiple, time-consuming and expensive tools: pelvic examination, transvaginal ultrasonography, PET-CT and laboratory tests.

Laboratory tests

A tumor marker is a naturally occurring molecule that is measured in serum, plasma, or other body fluids or in tissue extracts or paraffin-embedded tissues (to identify the presence of cancer) to assess patient prognosis or to monitor a patient´s

response to therapy with the goal of improving the clinical management of the patient. Tumor markers are found inside cells, both in the cytoplasm and nuclei, and they are associated with cell surface membranes. They also circulate in blood. The ideal marker for the purpose of diagnosis would have two characteristics: it would be secreted into the blood in measurable concentration only after the cells that produce it had undergone malignant transformation, and detection of it would permit conclusions as to the site of the tumor from which it arose. Unfortunately markers with close to 100% specificity (undetectable in benign diseases and healthy individuals) and 100% sensitivity (always detectable even in the early stages of a tumor) do not exist.

The most common laboratory test is CA125, the only ovarian cancer biomarker routinely used in clinical practice. In 1981, Bast et al. identified the CA125 antigen with the development of the OC 125 murine monoclonal antibody against cell line OVCA 433, which was derived from a patient with ovarian serous carcinoma. This new mucin molecule has been designated Ca125/MUC16 [mucin 16, cell surface associated *(MUC16)* gene] and consists of a 156–amino-acid tandem-repeat region in the*N*-terminus and a possible transmembrane region and tyrosine phosphorylation site in the *C*-terminus (2). The first immunoassay for CA125, commercialized in 1983. Assays for CA125 have since been adapted to automated platforms. Concentrations of CA125 may vary among manufacturers owing to differences in calibration, assay design, and reagent specificities. The lack of an International Standard for CA125 decreases progress in improving between-method comparability and manufacturers should specify the standard preparation against which their method is calibrated, and laboratories should indicate the CA125 method used on their clinical reports. It suffers from limited diagnostic performance due to poor sensitivity and specificity. A number of groups including the NACB 2008 the European Group on Tumor Markers (EGTM) and the National Institutes of Health (NIH) Consensus Conference have published guidelines for optimal use of tumor markers in routine clinical practice for screening, prevention, diagnosis, and treatment ovarian cancer (3,4,5). CA125 is recommended (in combination with transvaginal ultrasonography) forearly detection of ovarian cancer only in women at high risk for this disease and for differential diagnosis of suspicious pelvic masses in postmenopausal women. It is also recommended for monitoring treatment, prognosis, and disease relapse in patients with known ovarian cancer. It is not recommended for regular screening or diagnosis (3).

When ovarian cancer is detected at an early stage, where the disease is still contained within the ovaries (stage I), 5-year survival rates can approach 90% with optimal surgery and currently available combination chemotherapy. By contrast, ovarian cancer that has spread throughout the peritoneal cavity or outside the abdomen (stages III and IV) is associated with 5-year survival of less than 30%. In women with epithelial ovarian cancer, 80% have CA125 levels >35 kU/L, with elevations of 50%–60% in clinically detected stage I disease, 90% in stage II, and >90% in stages III and IV. Several benign pelvic conditions cause increased CA125. These include endometriosis, benign ovarian cysts, pelvic inflammatory disease and salpingitis, as well as nongynecologic diseases including cirrhosis, ascites, peritoneal inflammation, pleuritis/pericarditis, pancreatitis, renal failure, and liver disease. Also CA 125 increased during menstrual phase and descreased because regular smoking and caffeine consumption (6).

Human epididymis protein (HE4) was discovered by Kirchhoff et al in 1991 as a transcript exclusively expressed in distal epididymis in men (7). The gene, also known as WFDC2 is located on human chromosome 20q12-13.1, a region that includes several genes that encode whey acidic protein (WAP). Its mature 25-kDa glycosylated form consists of a single peptide and two whey acidic protein (WAP) domains that contain a 'four disulfide core' composed of eight cysteine residues. The function HE4 is unknown, but may be function as an antiproteinase within the male reproductive tract in sperm maturation. Human epidydimis protein (HE4) is glycoprotein overexpressed in patients with serous and endometrioid epithelial ovarian cancer (8-10). Since HE4 is overexpressed in ovarian cancers relative to normal tissues, 2003. Hellstrom et al examined the potential of HE4 as a secreted biomarker for ovarian cancer in serum. Murine monoclonal antibodies 2H5 and 3D8 were prepared against HE4 protein produced in mammalian cells from an HE4-IgG2a Fc fusion construct (11). A heterologous double

determinant immunoassay was established with the two antibodies that bound to distinct domains on the HE4 protein. The first commercially available assay for serum HE4 was developed as an EIA (Fujirebio Diagnostic, Inc., Malvern, PA). Today on the market there are many different manufacturers of commercial immunoassays (EIA, CMIA) to determinate HE4 in serum.

HE4 is a superior biomarker for distinguishing benign from malignant gynecological disease. The main challenge for laboratory tumor markers of ovarian cancer is to allow the accurate detection of malignancy as early as possible to imprive clinical outcome and survival of patients. The diagnostic accuracy of HE4 in differentiating malignant ovarian tumors from benign gynecological conditions was assessed by metaanalysis and found to have a pooled sensitivity of 0.74 and a pooled specificity of 0.87. Lin et al. searched the MEDLINE, EMBASE, and Cochrane Library databases for studies published up to June 2012 that evaluated HE4 accuracy. Meta-analysis was used to calculate sensitivity, specificity, the positive likelihood ratio (PLR), the negative likelihood ratio (NLR) and the area under curve (AUC). A total of 11 studies with 3395 patients who fulfilled all inclusion criteria were considered in the analysis. No publication bias was found. HE4 had a pooled sensitivity of 0.74 (95% confidence interval (CI), 0.72–0.76) and a pooled specificity of 0.87 (95% CI, 0.85–0.89). Overall, the positive likelihood ratio was 8.04 (95% CI, 4.89–13.21) and the negative likelihood ratio was 0.27 (95% CI, 0.22–0.34). When HE4 was combined with CA125, the sensitivity was higher than that of HE4 alone at the expense of lower specificity (12). Another meta-analyses in 2012 included 16 studies, to criticaly revise the availabile literature to confirm clinical value HE4. These meta-analyses revealed an ovarall sensitivity of 79% (95 Cl 76% to 81%) and specificity of 93% 95% CI 92% to 94%) for HE4, and an ovarall sensitivity of 78% (95% CI 76% to 80%) for CA125. Meta-analyses confirm that the risk for ovarian cancer is significantly increased for patients with HE4 positive results (OR 37,2), HE4 exhibited a significantly higher specifity than CA125 (93% vs. 78%) and HE4 outperforms CA125 in identifying ovrian cancer (LR+: 13,0 VS.4,2)(13).

CA 125 is elevated in benign gynecological conditions. HE4 is less frequently elevated than CA 125 in benign disease (8% vs 29%), improving specificity, particularly in premenopausal women. In endometriosis, CA 125 was elevated in 67% of cases, compared with 3% for HE4. Elevation of HE4 can occur in renal failure and in lung cancer (14-16).

A combination of CA 125 and HE4 was found to be a better predictor of malignancy than either marker alone. In a study assessing the performance of 65 ovarian cancer–related biomarkers for evaluation of adnexal masses, the CA 125–HE4 combination was found to be superior all other marker combinations (14). For the evaluation of possible malignant disease in women presenting with pelvic masses, a combination of CA 125, HE4, and age was found to provide a higher diagnostic value (area under the curve [AUC] of 0.797) than CA 125 alone (AUC of 0.677). This combination was also found to have diagnostic relevance in the setting where there is a need to distinguish endometrial cancer from benign uterine disease, with a sensitivity of 60.4% and a specificity of 100%. Various factors from malignancy may influence serum HE4 levels and should be carefully considered in interpreting values of HE4. Unlike CA125 levels, which decrease with age, HE4 levels increase significantly with age. HE4 levels are also affected by pregnancy: pregnant women have significantly lowered levels of HE4 in comparison with age-matched nonpregnant premenopausal women. Older women, women with a later menarche, and smokers also had significantly higher levels of HE4. Menstrual cycle, endometriosis and estrogen and progestin contraceptive usage do not alter serum levels of HE4 (17). With a molecular weight of 25 kD, which is below of the glomerular filtration cutoff, HE4 levels have also been found to be elevated in chronic kidney disease and renal failure.

The ROMA index combines CA125 and HE4 values along with the menopausal status into a predictive index, which in turn is used to calculate the predicted probability of ovarian cancer (from 0 to 100%). The regression formulae are as follows, where LN is the natural logarithm (16,17). In premenopausal women Predictive Index (PI) = −12.0 + 2.38 * LN (HE4) + 0.0626 x * LN (CA-125 In postmenopausal women Predictive Index (PI) = −8.09 + 1.04 * LN (HE4) + 0.732 * LN (CA-125 Predicted Probability (PP) = exp (PI) [1exp(PI)]. To calculate

the ROMA value, insert the calculated value for PI into following equation: ROMA value (%) = exp(PI) / [1 + exp(PI)] * 100 (18).

Several published studies show that ROMA index helps in the triage of pre - and postmenopausal women for ovarian cancer (19,20). Moore et al. found that the algorithm correctly classified 94% of women with epithelial ovarian cancer (18). This high accuracy helps to stratify the women into low- and high-risk groups and thus may contribute to better diagnosis, treatment and outcome.

Given the obsolescence guidelines dating back to 2008, European Group of Tumor Markers (EGTM) published 2012. Guidelines for Use of Biomarkers in Gynecological Cancer (21).

European Group of Tumor Markers (EGTM) conclusions are as follows:

Established tumor markers for ovarian cancer

- In women with epithelial ovarian cancer, approximately 80% have CA 125 levels > 35kU/L, with elevations in 50%-60% in clinical stage I disease, 80%-90% in stage II, and > 90% in stage III-IV.
- CA 125 is not recommended as a screening test in asymptomatic women without a hereditary risk outside the context of a clinical trial because CA 125 lacks diagnostic sensitivity for stage I disease and those with mucinous-type tumors and additionally lacks disease specificity, especially for premenopausal women.
- CA 125 is advised annually in women with a hereditary ovarian cancer syndrome in addition to pelvic and ultrasound examination. However, there is no evidence that screening these high-risk women reduces morbidity or mortality.
- CA 125 combined with Ultrasound is recommended in distinguishing benign from malignant disease in women with a pelvic mass, particularly in postmenopausal women. An algorithm to calculate the risk of malignancy index (RMI) has been developed, where CA 125 is incorporated with transvaginal ultrasound and menopausal status to estimate the probability of malignant potential for a pelvic mass in premenopausal and postmenopausal women with reported sensitivities of 71%-78 % and specificities of 75%-94 %.
- Concentrations of CA 125 > 95kU/L in postmenopausal women can discriminate malignant from benign pelvis masses with a positive predictive value of 95%.
- Benign conditions resulting in increased CA 125 levels are a confounding factor in premenopausal women e.g. (pregnancy, endometriosis, cysts and uterine leiomyoma).
- CA 125 may be considered for monitoring treatment of ovarian cancer, but there is no consensus on how to define a CA 125-based response. It is recommended that the marker response should be based on a decrement of 50% in concentrations or alternatively be based on a statistical estimation of decrements adjusted to both analytical and biological variation of the marker.
- CA 125 may be of prognostic significance preoperatively, postoperatively and during the first three courses of primary chemotherapy.
- CA 125 measurements are recommended during follow-up. Continuously elevated concentrations during follow-up are predictive for tumor growth. To indicate progression either a confirmed doubling of CA 125 levels or an approach based on analytical and biological variation may be used.
- Alpha-fetoprotein (AFP) and human chorion-gonadotropin (HCG) are established markers for germ cell tumors both for diagnosis and monitoring. Elevated AFP and HCG concentrations > 100 IU/L indicate the presence of non-dysgerminotous elements.

Potential tumor markers for epithelial ovarian cancer

- Human Epididymis protein (HE4) has been proposed as a new tumor marker especially for non-mucinous subtypes of epithelial ovarian cancer. Initial studies have suggested an increased diagnostic specificity of HE4 compared to CA 125, mainly in premenopausal women. The sensitivity of CA 125 and HE4 is suggested to be similar.
- Data indicate that HE4 measurement in healthy premenopausal women as well as in women with endometriosis may be carried out at any phase of the menstrual cycle, and irrespective of hormonal medication.
- An algorithm, the "Risk of Ovarian Malignancy Algorithm" (ROMA) has been suggested to evaluate the performance of utilizing the com-

bination of HE4 and CA 125 to predict the risk of serous epithelial ovarian cancer in women with pelvic mass. However, the ROMA algorithm should undergo further clinical evaluation before recommending the algorithm in clinical use.

- The utility of combing HE4 and CA 125 is unclear due to small and selected study populations. Further prospective studies are needed to investigate HE4 before implementing the marker into routine clinical practice.
- CEA and CA 19.9 measurements may be considered in determining treatment response in monitoring of patients with mucinous tumors.

In our laboratory, two years ago, we routinely measure CA 125 and HE4 on Cobas e411 device, Roche. Sample material is serum, collected using standard sampling tubes with separatinggel, after centrifugation of whole blood. Sample volume is 10 μL for HE4 and 20 μL for CA 125. Test principle HE4 is one-step sandwich assay, with measuring range 15-1500 pmol/L. CA 125 test is one-step sandwich assay with measuring range 0,6-5000 U/mL. The Elecsys CA 125 tumor marker assay is based on the monoclonal M11 and OC125 antibodies. Testing time is 18 min. We also calculated ROMA index and stratificate into low risk and high risk group. The following cut-off points were used in order to provide a specificity level of 75% for the Elecsys HE4 and Elecsys CA125 assay combonation: premenopausal women ROMA value ≥ 11.4% = High risk of finding epithelial ovarian cancer; ROMA value <11.4% = Low risk of finding epithelial ovarian cancer. In group postmenopausal women ROMA value ≥29.9% = High risk of finding epithelial ovarian cancer ROMA value <29.9% = Low risk of finding epithelial ovarian cancer (22). Also, in case of elevated urea and creatinine issue remark: Impaired kidney function can cause falsely elevated HE4.

CONCLUSION

The large challenge for laboratory tumor markers of ovarian cancer diagnosis is to allow the accurate detection of malignancy as early as possible to improve clinical outcome and survival of patients. Until recently, the only routinely used marker for ovarian, and unfortunatelly for the all others gynecological disease, was CA125. Determination of HE4 levels, together with those of CA125 and the calculation of the ROMA index, is a suitable method for improving primary detection of ovarian cancer. Combination of HE4 and CA125 is useful inassessing the response to treatment: correlation between radiology's imaging-techniques and tumor marker results was higher than 80%. Combination of HE4 and CA125 are useful in follow-up in 89,8% of all patients with ovarian carcinoma and in 95% of non-mucinous ovarian carcinoma. Combination of both tumor markers are recommended because changes in the relase pattern are frequently found with chemotherapy (23). The measurement of serum HE4 is a useful method for differential diagnosis between benign gynecologic disease and ovarian cancer. From literature data HE4 has also emerged as a serum biomarker for lung cancer, pulmonary adenocarcinoma, chronic kidney disease, renal failure and kidney fibrosis. Each of these conditions must be considered when interpreting HE4 levels in ovarian cancer. With a molecular weight of 25 kD, which is below the glomerular filtration cut-off, HE4 levels have also been found to be elevated in the urine of ovarian cancer patients compared with urine from healthy individuals or controls with benign disease. Pretreatment levels of HE4 have a prognostic value in ovarian cancer patients. 63,3% of pattients with positive HE4 before treatment have progression disease in the first five years of follow-up.

REFERENCES

1. Hrvatski zavod za javno zdravstvo, Registar za rak Republike Hrvatske. Incidencija raka u Hrvatskoj 2013., Bilten 38, Zagreb, 2015.
2. Bast RC, Feeney M, Lazarus H, Nadler LM, Colcin RB, Knap RC. Reactivity of a monoclonal antibody with human ovarian carcinoma. J Clin Invest. 1981;68: 1331-7.
3. Sturgeon CM, Duffy MJ, Stenman UH, et al. National Academy of Clinical Biochemistry laboratory medicine practice guidelines for use of tumor markers in testicular, prostate, colorectal, breast, and ovarian cancers. Clin Chem. 2008;54:e11– e79.
4. BonfrerJMG, DuffyMJ, RadtkeM, et al., Tumour markers in gynaecological cancers: EGTM recommendations Anticancer Res. 1999;19:2807–10.
5. NIH consensus conference, Ovarian cancer: screening, treatment, and follow-up. NIH Consensus Development Panel on Ovarian Cancer. JAMA. 1995;273:491–7.

6. Buamah P. Benign conditions associated with raised serum CA-125 concentration. J Surg Oncol. 2000;75: 264–5.
7. Kirchhoff C, Habben I, Ivell R, Krull N. A major human epididymis-specific cDNA encodes a protein with sequence homology to extracellular proteinase inhibitors. Biol Reprod. 1991;45:350–7.
8. Schaner E, Ross DT, Ciaravino G, et al., Gene expression patterns in ovarian carcinomas, Mol Biol Cell. 2003;14:4376–86.
9. Schwartz DR, KardiaSL, SheddenKA, et al. Gene expression in ovarian cancer reflects both morphology and biological behavior, distinguishing clear cell from otherpoor-prognosis ovarian carcinomas. Cancer Res. 2002;62:4722–29.
10. Drapkin R, von Horsten HH, Lin Y, Mok SC, Crum CP, Welch WR, et al. Human epididymis protein 4 (HE4) is a secreted glycoprotein that is overexpressed by serous and endometrioid ovarian carcinomas. Cancer Res. 2005;65:2162–69.
11. Hellstrom I, Raycraft J, Hayden-Ledbetter M, et al. The HE4 (WFDC2) protein is a biomarker for ovarian carcinoma. Cancer Res. 2003;63:3695–700.
12. Lin YL, Jin Bao Q, Sangvatanakul V. Human epididymis protein 4 for differential diagnosis between benign gynecologic disease and ovarian cancer: a systematic review and meta-analysis. Eur J Obstet Gynecol Reprod Biol. 2012;167:81-5.
13. Ferraro S, Braga F, Lanzoni M, Boracchi P, Biganzoli EM, Panteghini M. Serum Human Epididymis Protein 4 vs Carbohydrate Antigen 125 for Ovarian Cancer Diagnosis. J Clin Pathol. 2013;66(4):273-81.
14. Moore RG, McMeekin DS, Brown AK, et al. A novel multiple marker bioassay utilizing HE4 and CA125 for the prediction of ovarian cancer in patients with a pelvic mass. Gynecol Oncol. 2009;112:40–6.
15. 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;110:374–82.
16. Moore RG, Brown AK, Miller MC, et al. The use of multiple novel tumor biomarkers for the detection of ovarian carcinoma in patients with a pelvic mass. Gynecol Oncol. 2008;108:402–8.
17. Moore RG, Miller MC, Eklund EE, Lu KH, Bast RC Jr, Lambert-Messerlian G. Serum levels of the ovarian cancer biomarker HE4 are decreased in pregnancy and increase with age. Am J Obstet Gynecol. 2012; 206(4):349.e1-7.
18. Moore RG, Jabre-Raughley M, Brown AK, et al. Comparison of a novel multiple marker assay vs the risk of malignancy index for the prediction of epithelial ovarian cancer in patients with a pelvic mass. Am J Obstet Gynecol. 2010;203:228e1–e6.
19. Montagnana M, Danese E, Ruzzenente O, et al. The ROMA (Risk of Ovarian Malignancy Algorithm) for estimating the risk of epithelial ovarian cancer in womenpresenting with pelvic mass: is it really useful? Clin Chem Lab Med. 2011;49:521–5.
20. Molina R, Escudero JM, Auge JM, et al. HE4 a novel tumour marker for ovarian cancer: comparison with CA125 and ROMA algorithm in patients with gynaecological diseases. Tumour Biol. 2011;32:1087–95.
21. http://www.egtm.eu/professionals/gynecological_cancer Accessed on 21.01.2016.
22. http://www.cobas.com/home/product/clinical-and-immunochemistry-testing/elecsys-human-epididymal-protein-4.html Accessed on 21.01.2016.

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