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ORIGINAL PAPER

Comparison of Specific Ovarian Tumor Markers by Elecsys Analyzer 2010

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ABSTRACT

Background: the most widely used tumor marker in ovarian cancer, often considered the 'gold standard' is CA125 but reliable clinical evidence demonstrates that human epididymis protein (HE4), used alone or in combination with CA125, substantially improves the accuracy of screening and/or disease monitoring. Aim: to evaluate the reliability of the determination a tumor marker HE4 in comparison with CA125 on the Elecsys analyzer 2010 in epithelial ovarian cancer, benign ovarian cyst and healthy controls. **Methods**: we prospectively determined CA125 and HE4 serum levels in the Biochemical-Immunological-Haematological "Medical Laboratory" Ilidza, Sarajevo, B&H between June 1st and December 31st 2011. Electro-chemiluminescence immunoassay (ECLIA) methods for quantitative determination in vitro were performed on the Roche/Hitachi Elecsys 2010 Immunoassay Analyzer. Standard methods of descriptive statistics were performed for the data analysis. **Results:** univariate statistical analyze of tumor marker control serum revealed a high reliability for both CA125 and HE4 determination (p>0.05). Levey-Jennings charts of quality control data show that the target and the obtained values of both markers control sera do not differ significantly in relation to the ideal value. In a total number of 60 patients compared values of tumor markers show a high correlation (r=0.85). This study confirmed higher sensitivity and specificity of HE4 tumor marker compared with CA125. ROC-AUC values show that the diagnostic performance of HE4 was significantly higher compared with CA125. **Conclusion:** We concluded that HE4 was better than CA125 as a single tumor marker.
Key words: ovarian tumor marker, CA125, HE4

1. INTRODUCTION

Noninvasive biomarker testing is essential for practical general population monitoring. Ovarian cancer is a lethal gynecologic malignancy with five-year survival of only 20% to 40% for advanced stage disease. Detection at an early stage would likely have significant impact on mortality rate. The most widely used tumor marker in ovarian cancer, often considered the 'gold standard' is CA125 (1). Measurement of CA125 can be performed with different commercial assays resulting in a certain degree of variation. Biomarker development efforts to date clearly indicate that no individual biomarker, including CA125, can provide sufficient sensitivity at high specificity for the early detection of ovarian cancer. CA125 can be elevated in a number of conditions unrelated to ovarian cancer, resulting in decreased specificity.

When values below 35 U/mL are designated as normal, CA125 is elevated in 80% of epithelial ovarian cancers (2). CA125 is elevated in approximately 50%-60% of stage I epithelial ovarian cancers and 75%-90% of patients with advanced stage disease (3, 4).

The sensitivity of CA125 to identify early stage disease is limited as a screening tool. The quest for other biomarker candidates has continued because a single CA125 value at a given time point will not reach a specificity of 99.6%, and approximately 20% of ovarian cancers may not express this antigen. Therefore, it is necessary to identify additional informative biomarkers that complement CA125. Reliable clinical evidence demonstrates that human epididymis protein (HE4), used alone or in combination with CA125, substantially improves the accuracy of screening and/or disease monitoring. HE4, found primarily in the epithelia of normal genital tissues is elevated in epithelial ovarian cancer (5,6). HE4 has greater specificity in the premenopausal age group than CA125 given it does not appear to be expressed at high levels in the setting of benign conditions (7-9).

In a systemic review of women with suspected gynecologic disease HE4 demonstrated a higher specificity (93% *vs* 78%) and similar sensitivity (79%) to CA125 when distinguishing benign disease from ovarian cancer (10).

Studies have demonstrated a potential benefit in combining HE4 and CA125 when quantifying risk potential malignancy in the evaluation of a pelvic mass (11, 12). Even with new technology, it is unlikely that an individual biomarker will reach a specificity of 99.6%, positive predictive value of 10%, and sensitivity greater than 75% when screening an asymptomatic general population.

It is important to measure the concentration of the tumor marker by the same method. Different antibodies, matrix and calibrator which are used in different methods may give different results. This means that different commercial tests give results for tumor markers that are not mutually comparable (13). The aim of this study was to evaluate the reliability of the determination a tumor marker HE4 in comparison with CA125 on the Elecsys analyzer 2010 in epithelial ovarian cancer, benign ovarian cyst and healthy controls.

2. PATIENTS AND METHODS

From June to December 2011, 60 patients were included in a prospective study conducted at the Biochemical-Immunological-Haematological "Medical Laboratory" Ilidza, Sarajevo, Bosnia and Herzegovina.

Study group (n=60) was consisted of 28 premenopausal and 32 postmenopausal patients which were previously diagnosed as some type of epithelial ovarian cancer (n=20), benign ovarian cyst (n=20) or were with normal woman`s gynecological results (n=20).

Samples of venous blood were collected in serum gel tubes. After centrifugation and separation of serum, all samples were frozen until analysis. CA125 and HE4 serum levels were determined by Electro-chemiluminescence immunoassay (ECLIA) method for quantitative determination in vitro. Assays were performed on the Roche/Hitachi Elecsys 2010 Immunoassay Analyzer by Roche Diagnostics Ltd., Switzerland. All assays were run according to manufacturer's instructions, and appropriate controls were within the ranges provided by the manufacturer for all runs.

The Elecsys CA 125 II tumor marker assay is based on the monoclonal M 11 and OC 125 antibodies from Fujirebio Diagnostics, Inc. Every Elecsys reagent set has a barcoded label containing specific information for calibration of the particular reagent lot. The predefined master curve is adapted to the analyzer using the relevant CalSet CA125 II. The measurement was performed by the generated voltage induced from chemi-luminescent emission of the photomultiplier. Values were expressed in units per milliliter (U/mL). Measuring range was from 0.600-5000 U/mL (defined by the lower detection limit and the maximum of the master curve). Test duration was 18 min. The reference value was <35 U/ml. Sensitivity (the minimum detectable dose of CA125) was determined to be 0.6 U/ml. Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluted buffer). The following material was required for the analysis: the serum sample, Test Reagent CA125 II, CalSet CA125 II, PreciControl Tumor Marker1 (TM1) and PreciControl Tumor Marker2 (TM2), Diluent Universal, Procell and Cleancell.

The Elecsys Electro-chemiluminescence immunoassay (ECLIA) for the quantitative determination of human epididymal protein 4 (HE4) in serum and plasma assay is a two-step sandwich immunoassay. In this study two mouse monoclonal antibodies (2H5 and 3D8) directed against two epitopes in the C-WFDC domain of HE4 were used. First, sample was incubated with a biotinylatd monoclonal HE4-specific antibody and a monoclonal HE4-specific antibody labeled with a ruthenium to forms a sandwich complex. After addition of streptavidin-coated microparticles, the complex bounds to the solid phase via interaction of biotin and streptavidin. The reaction mixture is aspirated into the measuring cell where the microparticles are magnetically captured onto the surface of the electrode. Unbound substances are then removed. A voltage is applied to the electrode to induce chemiluminesent emission which is measured by a photomultiplier. Test duration was 18 min. The results are determined via a calibration curve that is instrument-specifically generated by 2-point calibration and a master curve provided via the reagent barcode. Measuring range was from 15–1500 pmol/L. The serum sample, HE4 Test Reagent, HE4 CalSet, PreciControl HE4 Tumor Marker1 (TM1) and PreciControl HE4 Tumor Marker2 (TM2), Diluent Multi Assay, Procell and Cleancell.

All reagents were supplied by Roche Diagnostics Ltd. Target values and approximate target ranges of the reconstituted calibrators are presented in Table 1. and Table 2.:

Control serum	Target range (value) U/mL
PreciControl TM1- CA125	27.1 – 41.5 (34.3)
PreciControl TM2- CA125	112– 136 (124.00)

Table 1. *CA125–approximate target ranges and target values*

Control serum	Target range (value) pmol/L
PreciControl TM1 – HE4	32.9 – 51.2 (45.0)
PreciControl TM2–HE4	258 – 450 (354)

Table 2. *HE4–approximate target ranges and target values*

The precision of the method was determined using twenty control serum samples for each PreciControl tumor marker. The results are summarized in the following Table 3.

CA125U/mL	HE4 pmol/L		
TM1	TM2	TM1	TM2
20 samples	20 samples	20 samples	20 samples
Variance=0.3512	Variance=0.1973	Variance=0.1066	Variance=0.1547
SD = 0.5926	SD = 0.4442	SD = 0.3266	SD = 0.3934
accept Normality (P=0.5285) (χ^2=1.275; df=2)	accept Normality (P=0.307) (χ^2=2.362; df=2)	accept Normality (P=0.5256) (χ^2=1.333; df=2)	accept Normality (P=0.3625) (χ^2=2.029; df=2)

Table 3. *Chi- square Test for Normal Distribution of the investigated tumor markers determination methods precision*

Statistical analysis was performed with SYSTAT 13.1 statistic software (Systat Software Inc., San Jose, California). Standard methods of descriptive statistics were performed for the data analysis. For the purposes of analysis in this study, the percentage of true positive (TP), true negative (TN), false positive (FP) and false negative results (FN), standard deviation, sensitivity (TP/TP+FN), specificity (TN/FP+TN) were used to evaluate the diagnostic performance of both markers. The diagnostic performance was studied with ROC (Receiver Operating Characteristic) curves based on continuous variables. The area under curve (AUC), standard error (SEAUC), and confidence interval (CIAUC) for AUC were calculated according to the nonparametric method of DeLong et al. (14). This method was used to compare AUCs considering the fact that measurements of HE4 and CA125 were done for the same objects (group of patients). The level of significance was taken as $p<0.05$.

3. RESULTS

Univariate statistical analyze of tumor marker control serum revealed a high reliability for both CA125 and HE4 determination ($p>0.05$). It was found that the method of determining on an Elecsys 2010 analyzer for both tests showed a satisfactory degree of reproducibility. Chi-square test for normal distribution showed accepting values in all control sera. Levey-Jennings charts of quality control data show that the target and the obtained values of both markers control sera do not differ significantly in relation to the ideal value (Figure 1- 4.).

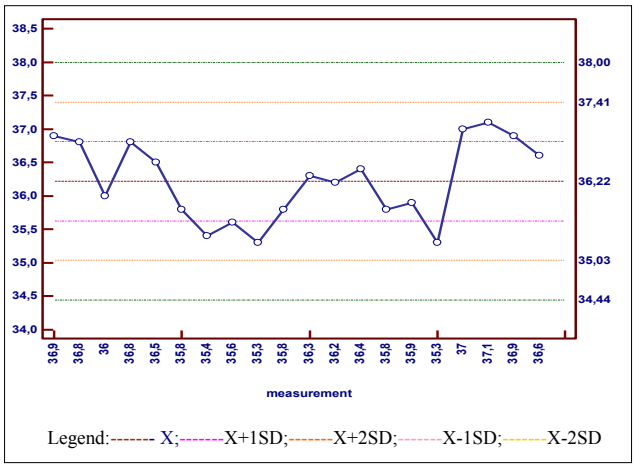

Figure 1. *Levey-Jennings chart of TM1 CA125 test*

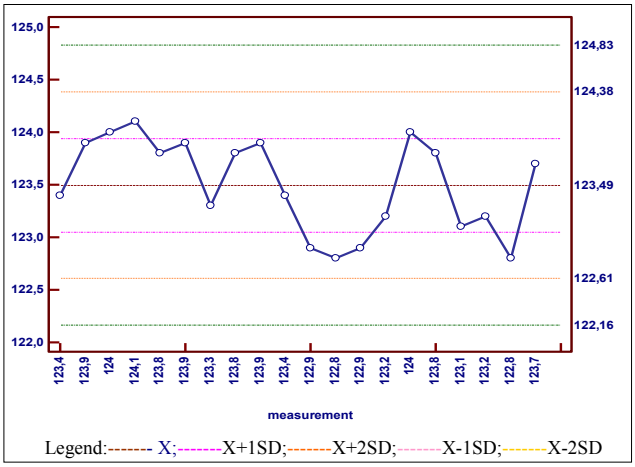

Figure 2. *Levey-Jennings chart of TM2 CA125 test*

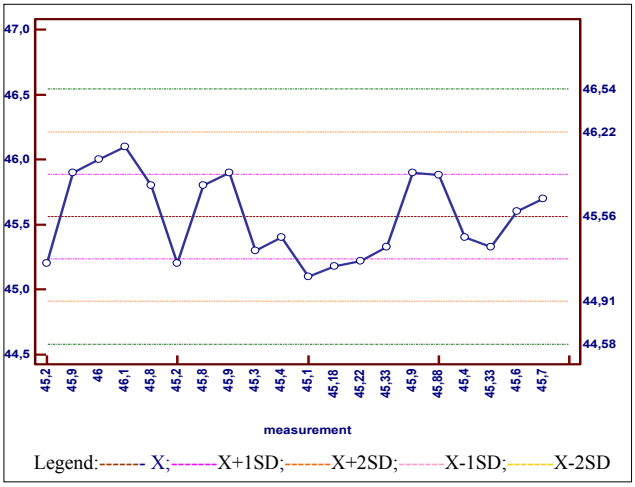

Figure 3. *Levey-Jennings chart of TM1 HE4 test*

Compared values of investigated patients tumor markers show a high correlation (r=0.85).

Study group (n=60) was consisted of three groups (n=20 each) of patients which were previously diagnosed as follows:

- Patients with normal woman`s gynecological results (group I–control group)
- Patients with some type of ovarian cancer (group II)
- Patients with benign ovarian cyst (group III)

Table 4. show range and mean values of tumor markers in investigated groups of patients.

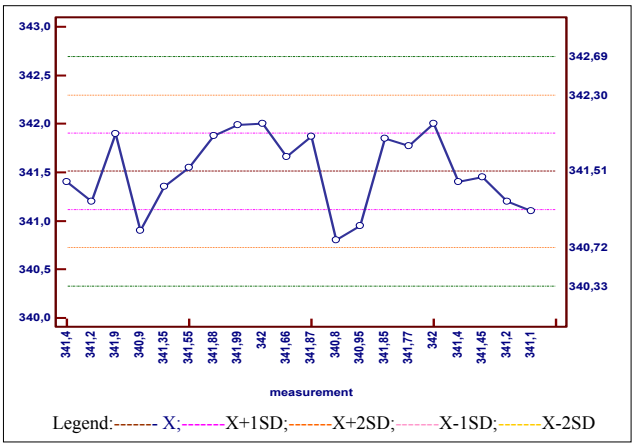

Figure 4. *Levey-Jennings chart of TM2 HE4 test*

Group:	CA125 U/mL min-max (mean)	HE4 pmol/L min-max (mean)
I	3.54–32.2 (14.31)	47.39 – 102.5 (65.13)
II	14.17-937.4 (60.97)	34 – 1383 (84.66)
III	8.95 – 59.1 (18.8)	28.6 – 103.6 (62.5)

Table 4. *Tumor marker range and mean values in studied groups*

By comparing the CA125 and HE4 tumor markers values in the studied groups we found that in control group I there were not differences between CA125 and HE4, in group II there were 60% CA125 and 95% HE4 positive values and in the group III there were only 25% CA125 borderline test values (Table 5).

Values	Group I		Group II		Group III	
	CA125	HE4	CA125	HE4	CA125	HE4
Normal	20/20 (100%)	**20/20 (100%)**	8/20 (40%)	1/20 (5%)	15/20 (75%)	20/20 (100%)
Borderline	0/20 (0%)	0/20 (0%)	0/20 (0%)	0/20 (0%)	5/20 (25%)	0/20 (0%)
High	0/20 (0%)	0/20 (0%)	12/20 (60%)	**19/20 (95%)**	0/20 (0%)	0/20 (0%)

Table 5. *Comparison of the tumor markers CA125 and HE4 values in the studied groups*

This study confirmed higher sensitivity and specificity of HE4 tumor marker compared with CA125 (Table 6).

Tumor marker test characteristics:	CA125	HE4
Prevalence	12/10000 = 0.0012 (0.12%)	19/10000 = 0.0019 (0.19%)
Sensitivity	12/20 = 0.65 (60%)	19/20 = 0.95 (95%)
Specificity	48/40 = 0.851 (83.33%)	41/40 = 0.975 (97.5%)
Positive predictive value	12/60 = 0.2 (20%)	19/60 = 0.317 (31.7%)
Negative predictive value	48/60 = 0.8 (80%)	41/60 = 0.683 (68.3%)

Table 6. *Comparison of CA125 and HE4 tumor marker diagnostic performance*

Tumor marker:	premenopausal	postmenopausal
CA125	0.466 (0.2401-0.6414)	0.71 (0.60-0.82)
HE4	0.672 (0.5193-0.837)	0.93(0.86-0.97)

Table 7. *AUC (95% CI) values of studied tumor markers in premenopausal and postmenopausal patients*

AUC (Area Under Curve) values, determined by ROC characteristics, show that the diagnostic performance tumor

marker HE4 was significantly higher than the tumor marker CA125 in both premenopausal and postmenopausal patients (Table 7).

4. DISCUSSION

With the exception of highly invasive procedures such as biopsy and surgery, the evaluation of circulating biomarkers offers the most definitive means of distinguishing benign from malignant cases. Several recent studies have evaluated various panels of circulating biomarkers in ovarian cancer patients and benign cases. Our study aimed to investigate the performance of serum tumour markers CA125 and HE4. Based on the obtained values of tumor markers and the actual condition in the test group, we determined the diagnostic value of both tumor markers. Sensitivity of CA125 was 60% with specificity of 83.33%, which was significantly less than the sensitivity of HE4 of 95% and it´s specificity of 97.5%. Positive predictive value of CA125 was 20%, while 31.7% of the HE4. The negative predictive value of tumor marker CA 125 was 80%, versus 68.3% of the HE4. Our study confirmed that the sensitivity and specificity of HE4 were significantly higher than the CA125 tumor marker ($p = 0.047$) which is consistent with research of Nolen and colleagues (11). Because of false positive CA125 values in benign gynecological tumors Moore et al (2008) also measured the serum levels of HE4, to increase the sensitivity and specificity of the diagnosis of this disease. They concluded that HE4 was better single marker to detect disease at an early stage, and that the parallel monitoring of both markers can better assess the risk of malignancy and may provide valuable information in distinguishing ovarian cancer from endometrioid cysts (12). Park et al. (15, 16) compared diagnostic performance of CA125 and HE4 in various gynecologic and non-gynecologic diseases. Their conclusions were: HE4 demonstrated comparable diagnostic performances to CA125, though each marker had its own strengths and weaknesses and combining CA125 and HE4 might be more advantageous than either one alone. The sensitivities and specificities of CA 125 and HE4 observed in our study are very similar to those observed by Moore et al. (12, 17), as is the observation that the two biomarkers display diagnostic complementation as each improves upon the discriminatory power of the other.

5. CONCLUSION

In our study, the ROC-AUC values for CA125 were significantly lower compared with HE4, suggesting a significantly higher performance of HE4 and we concluded that HE4 was better than CA125 as a single tumor marker. In summary, our validation study was able to demonstrate similar performance indices as those recently published in the literature.

CONFLICT OF INTEREST: NONE DECLARED.

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