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HE4 IN COMPARISON WITH OTHER BIOMARKERS IN OVARIAN CANCER DIAGNOSTICS

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Ovarian cancer is the leading cause of gynecological cancer death, representing 5% of all cancers in women and 23% of gynecological cancer. Ovarian cancer has a poor prognosis, mainly because of the late detection. Mortality, despite the decline in the last 10 years, is still very high. Worldwide attention is therefore focused on the potential research and the subsequent treatment of this cancer (1, 2).

The first aim of our study was to evaluate if human epididymis protein 4 (HE4) is a useful biomarker and broadens the possibilities in ovarian cancer diagnostics. The second aim was to evaluate the benefits of each biomarker of our panel for the ovarian cancer diagnostics. We compared the results of following tumor markers: cancer antigen 125 (CA 125), HE4, cancer antigen 19-9 (CA 19-9), cancer embryonic antigen (CEA), thymidinkinase (TK), tissue polypeptidic antigen (TPS) and tissue polypeptidic antigen (MonoTotal).

MATERIAL AND METHODS

The total number of females in our study was 266. We divided the patients into two groups. The age characteristic of both groups is shown in the Tab. 1. The first group consisted of 19 females with ovarian cancer with equal representation of stages FIGO I–IV. Second group included 247 patients with benign diseases (ovarian cysts, myomas, endometrial polyps). Serum samples were collected prior to surgery or any other form of treatment. All cancer diagnoses were histologically verified.

The serum samples were analyzed at the Laboratory of Immunoanalysis, Faculty of Medicine in Pilsen, (Czech Republic) from March 2010 to January 2012. Samples of venous blood were collected using the VACUETTE blood collection system (Greiner Bio-one Company, Kremsmünster, Austria). Blood was centrifuged for 10 minutes at 1700 ×g. Serum samples were immediately frozen to –80 °C. Samples were thawed only once, just prior to analyses. Serum levels of CA 125, CEA and CA 19-9 were measured using a DxI instrument (Beckman Coulter, Brea, California, USA). Serum levels of HE4 were measured using an enzyme immunometric assay kit (Fujirebio Diagnostics, Göteborg, Sweden). TK was measured using radioisotope assay kit (Immunotech, Prague, Czech Republic). TPS and MonoTotal were measured using IRMA radioisotope assay kits (IDL Biotech, Bromma, Sweden).

Tab. 1 Age characteristic of the patient groups

Diagnosis	Count (N)	Age (years)			
		Mean	Median	Min.	Max.
Ovarian cancer	19	65.63	62	43	84
Benign ovarian tumor	247	61.24	54	33	79

Tab. 2 Ovarian cancer *vs.* benign tumor

Parameter (units)	Diagnosis	N	Mean	Median	Range	*p-Value* Wilcoxon test
CA 125 (kIU/l)	**Cancer**	19	1669.00	1725.00	54.00 – 4621	<0.0001
	Benign	247	27.50	14.00	23.00 – 1244	
HE4 (pmol/l)	**Cancer**	19	595.06	421.9	50.87 – 3266	<0.0001
	Benign	247	80.24	52.70	23.00 – 1570	
MonoTotal (IU/l)	**Cancer**	19	626.9	501.7	710.9 – 2844	<0.0001
	Benign	247	79.50	49.90	5.00 – 2255	
TPS (IU/l)	**Cancer**	19	309.1	144.0	25.00 – 1453	<0.0001
	Benign	247	83.27	46.00	10.00 – 1226	
TK (IU/l)	**Cancer**	19	11.11	9.50	3.50 – 24.10	0.3022
	Benign	247	9.56	5.90	2.50 – 29.80	
CA 19-9 (kIU/l)	**Cancer**	19	23.00	11.00	1.00 – 124.0	0.3060
	Benign	247	16.27	8.00	1.00 – 428.0	
CEA (µg/l)	**Cancer**	19	25.00	5.30	2.50 – 225.0	0.1471
	Benign	247	1.83	1.20	0.50 – 20.60	

CA 125 (cancer antigen 125), HE4 (human epididymis protein 4), MonoTotal (tissue polypeptidic antigen), TPA (tissue polypeptidic antigen), TK (thymidinkinase), CA 19-9 (cancer antigen 19-9), CEA (cancer embryonic antigen)

The SAS 9.2 (Statistical Analysis Software release 9.2; SAS Institute Inc., Cary, NC, USA) was used for all statistical analysis. A summary of statistical findings for age and serum levels of each of the analytes was presented. The Wilcoxon test was used to compare distributions of values between benign and malignant tumors.

RESULTS

Comparing the parameters of serum level markers between the benign and malignant groups of patients a statistically significant differences were found in the following biomarkers: CA 125, HE4, MonoTotal and TPS ($p < 0.0001$ for each analyte). CA 19-9, TK and CEA were not significant. All the results are shown in Tab. 2.

Tab. 3 shows the analytical parameters of all biomarkers which were used in the study. We have evaluated cut-off, sensitivity, positive predictive value (PPV) and negative predictive value (NPV) at 95% specificity. We have calculated area under the curve (AUC) and the biomarkers in Tab. 2. and Tab. 3. are ranked according this parameter. The highest level of AUC was achieved for CA 125 (AUC = 0.987), the second highest level was achieved for HE4 (AUC = 0.907) and the lowest level was achieved for CEA (AUC = 0.483).

DISCUSSION

Tumor markers are currently used for the follow-up and therapy effect monitoring. In evaluating data, we have focused on the possibilities of using selected biomarkers in ovarian cancer diagnostics. Our panel of biomarkers consisted of the traditional tumor markers (CA125, CA19-9, CEA) which have been used in relations to the ovarian cancer for a long time. Then we evaluated a relatively new marker HE4, which we started to measure in 2010. In addition we have filled in the biomarker panel with the proliferative tumor markers from the group of cytokeratins (TPS, MonoTotal) and non-specific tumor marker TK.

CA 125 determination in combination with ultrasonography was used in the past for the diagnosis of ovarian cancer (3, 4). A major disadvantage of CA 125 is that up to 20% of ovarian cancers lack expression of this antigen. The second disadvantage is a low specificity of CA 125. Abnormal serum levels of CA 125 may be observed in several benign and malignant diseases (5, 6). It is therefore necessary to combine CA 125 with the other tumor markers to provide a better diagnostic efficiency.

The combination of CA125 and HE4 improves the results achieved by CA125 alone. About 20% of epithelial ovarian cancer show a slight elevation of CA125. For more than 50% of these malignancies, elevated levels of HE4 can be observed, and combinations of these markers may therefore optimize the potential for a successful diagnosis of ovarian malignancy in these patients. Another factor supporting a combination of both markers is that elevated levels of CA125 can be observed as a result of physiological conditions such as menstruation or pregnancy, as well as in benign ovarian tumor, inflammation and the presence of endometriosis and fibroids. This false positivity in the group of premenopausal patients may cause problems in routine clinical practice. Therefore, a combination of HE4 and CA125 increases specificity and sensitivity of testing in ovarian cancer diagnostics.

Using of biomarker HE4 as a single test in ovarian cancer testing is also problematic. Mucinous ovarian cancer has almost no expression of HE4. HE4 serum levels are very low or negative. HE4 is overexpressed in serous and endometroid histotype of the ovarian cancer. Preliminary studies of HE4 reported a higher specificity than CA 125 in different benign and malignant conditions, excluding renal failure (7). Patients with renal failure had very high HE4 serum levels. Patients with this disease were excluded from our study. The major part of the studies in serum has been already published that HE4 sensitivity and specificity were better than CA 125 (8–12). Our results didn't confirm this fact. If we consider our results, we see that the best values were achieved for CA125 followed by HE4.

Tab. 3 Analytical parameters of individual analytes at specificity 95%

Analyte (units)	**CA 125** (kIU/l)	**HE4** (pmol/l)	**MonoTotal** (IU/l)	**TPS** (IU/l)	**TK** (IU/l)	**CA 19-9** (kIU/l)	**CEA** (µg/l)
AUC	**0.987**	**0.907**	**0.836**	**0.755**	**0.676**	**0.573**	**0.483**
Cut-off	70.000	124.100	231.100	248.700	17.000	43.000	4.600
Sensitivity	89.500	73.330	63.280	36.810	22.280	16.710	11.100
PPV	50.000	50.180	63.160	29.210	18.280	15.840	11.170
NPV	99.000	97.510	97.900	96.520	96.000	95.400	95.000

AUC: area under the curve; PPV: positive predictive value; NPV: negative predictive value

Our data are consistent with the second group of the studies with the higher sensitivity of CA 125 than HE4 (13, 14).

Cytokeratins were included in our panel because significantly elevated serum levels of TPS were found in serum samples from patients who had ovarian carcinoma compared with patients who had benign tumors (6, 15).

Tumor markers CA 19-9, TK and CEA didn't show statistically significant different serum levels in the group of malignant tumors, compared to other benign ovarian diagnoses. These two markers are often elevated in relation to the benign or malignant disease of the gastrointestinal tract and therefore they are not directly related to gynecological diagnosis. CA 19-9 can be useful as an additional parameter in diagnostics of the mucinous type of ovarian cancer. In this case is the CA 19-9 elevated (16). However, their ability to distinguish between benign and malignant tumor is limited. Elevated serum levels may be found in benign mucinous tumors as well as in malignant tumors (17). In conclusion, determination of HE4 levels, together with CA125 improves a primary detection of ovarian cancer and broadens the range of differential diagnostic possibilities for distinguishing between malignant and benign tumors. MonoTotal and TPS confirmed their status of markers of proliferation and can be used to the monitoring the activity and aggressiveness of the tumor. Tumor markers CA 19-9, TK and CEA didn't show statistically significant different results.

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SUMMARY

Ovarian cancer is the leading cause of gynecological cancer death. The first aim of our study was to evaluate if HE4 broadens the possibilities in ovarian cancer diagnostics. The second aim was to evaluate the benefits of each biomarker of our panel. We compared the results of following tumor markers: CA 125, HE4, CA 19-9, CEA, TK, TPS, MonoTotal.

The total number of females in our study was 266. We divided the patients into two groups. The first consisted of 19 females with ovarian cancer and the second of 247 females with benign ovarian tumors. Serum samples were collected prior to surgery or any other form of treatment.

Significant difference between the benign and malignant group was found in following biomarkers: CA 125, HE4, MonoTotal and TPS. CA 19-9, TK and CEA were not significant. We have evaluated cut-off, sensitivity, positive predictive value and negative predictive value at 95% specificity and area under the curve (AUC). The highest level of AUC was achieved for CA 125 (AUC = 0.9951), the second highest level (AUC = 0.9534) was achieved for HE4 and the lowest level (AUC = 0.5324) was achieved for CEA marker. In conclusion determination of HE4 levels, together with CA125 improves a primary detection of ovarian cancer. MonoTotal and TPS confirmed their status of marker of proliferation and they can be used for the monitoring the activity and aggressiveness of the tumor. Tumor markers CA 19-9, TK and CEA didn't show statistically significant different results.

HE4 v porovnání s ostatními biomarkery v diagnóze rakoviny vaječníku

SOUHRN

Rakovina vaječníku je nejčastější příčinou úmrtí v oblasti zhoubných gynekologických onemocnění. Cílem naší studie bylo v první řadě zhodnotit, zda vyšetření HE4 je schopno rozšířit možnosti v diagnostice karcinomu vaječníku. Naším dalším cílem bylo zhodnotit přínos jednotlivých biomarkerů námi vybraného panelu. V naší práci jsme porovnali výsledky těchto nádorových markerů: CA 125, HE4, CA 19-9, CEA, TK, TPS a MonoTotal. Celkový počet žen v naší studii byl 266. Soubor jsme rozdělili do dvou skupin. První skupina se skládala z 19 žen s rakovinou vaječníku a druhá z 247 žen s benigními ovariálními tumory. Vzorky séra byly odebrány před operací nebo zahájením jiné formy léčby. Statisticky významný rozdíl mezi benigní a maligní skupinou byl nalezen v hodnotách následujících biomarkerů: CA 125, HE4, MonoTotal a TPS. Rozdíly v hodnotách CA 19-9, CEA a TK nebyly statisticky významné. Zhodnotili jsme cut-off, citlivost, pozitivní prediktivní hodnotu (PPV) a negativní prediktivní hodnotu (NPV) při 95 % specificitě a plochu pod křivkou (AUC).Nejvyšší úrovně AUC bylo dosaženo u CA 125 (AUC = 0,9951), druhé nejvyšší úrovně (AUC = 0,9534) bylo dosaženo u HE4 a nejnižší hladina (AUC = 0,5324) byla naměřena u CEA. Závěrem je možná říci, že stanovení HE4, spolu s CA125 zlepšuje primární detekci rakoviny vaječníku. Nádorové markery MonoTotal a TPS potvrdily svůj status markerů proliferace a mohou být použity pro sledování růstu a agresivitu nádoru. Nádorové markery CA 19-9, TK a CEA nevykazovaly statisticky významně odlišné výsledky.

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