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Diagnostic performance of the biomarkers HE4 and CA125 in type I and type II epithelial ovarian cancer☆

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HIGHLIGHTS

- With HE4 and CA125 we can diagnose the aggressive type II EOC at all stages and ages most correctly.
- The diagnostic safety for the dual markers HE4 and CA125 is not acceptable in early stage type I EOC.
- Our results support that EOC should be looked upon as several different diseases.

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ABSTRACT

Objective. To evaluate the diagnostic performance of HE4 and CA125 in patients presenting with suspicious malignant ovarian cysts. We especially wanted to investigate the levels of HE4 and CA125 with regard to the gene and histology-unifying model of type I and type II epithelial ovarian cancer (EOC).

Methods. Plasma from 373 women presenting with a suspicious malignant ovarian cyst was collected prior to surgery. Histology, grade, and stage were determined according to FIGO-classification. HE4 and CA125 were analyzed using ELISA, and the markers were evaluated for significance separately and in combination. Receiver operating curves, the area under the curve, sensitivity and specificity were estimated.

Results. The combination of HE4 and CA125 resulted in the best diagnostic power in comparing benign tumors to EOC (ROC AUC 0.93, sensitivity 94.4% at 75% specificity) for type II. Diagnostic power in type I (ROC AUC 0.79, sensitivity 61.9% at 75% specificity) was less impressive. In particular, mucinous benign vs. malignant tumors could not significantly be separated by the dual marker combination. Impressively high ROC AUC 0.99 was found for the late stage type II EOC with 100% sensitivity at 75% specificity.

Conclusions. HE4 and CA125 have a good ability to diagnose the more aggressive type II tumors but a poor diagnostic ability when patients are presenting with slow-growing type I in the early stage. Our results support the hypothesis that EOC should be looked upon as several different diseases, and that we lack biomarkers for subgroups of EOC.

Introduction

Because of the great heterogeneity in molecular and biological status, epithelial ovarian cancer (EOC) is actually many different diseases, which have different clinical outcomes and may require different treatments [1]. The four major histological subtypes are, based on their morphologic features, serous, endometrioid, clear cell, and mucinous. Traditionally EOC has been thought to arise from epithelial cells that cover the ovary's surface and even more frequently line subserosal cysts. Recent studies indicate that EOC also arises from the fallopian tube epithelium and from the endometrium via retrograde menstruation [2–4]. EOC is the most lethal malignancy with gynecologic origin in the Western world. The lack of clearly identified precancerous lesions, reliable screening tests, and unspecific early symptoms results in late diagnosis. When detected at an early stage (25%) the disease is highly curable [5].

Based on morphological and molecular genetics, a novel tumor origination and progression model was proposed, which divided EOC into type I and type II tumors [3,6,7]. Type I tumors are suggested to behave in an indolent manner and are more often confined to the ovary at diagnosis, with a stable genome and without TP53 mutations, although somatic mutations are frequently detected in a number of genes [8]. Type II tumors are suggested to be more aggressive and genetically highly instable; the majority have TP53 mutations and almost half of

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the cases have mutation, hypermethylation, or dysfunction of BRCA1/2 [9]. These aggressive tumors account for 75% of all EOC and are responsible for 90% of deaths from the disease [7,10].

In September 2011, the FDA cleared two glycoproteins, human epididymis protein 4 (HE4) and carbohydrate antigen (CA125), to be used together to estimate the risk for EOC in women with a pelvic mass. Our group and others have evaluated the marker combination, and found HE4 to be complementary to CA125 [11,12]. CA125 has been used in ovarian cancer diagnosis for 30 years and is elevated in approximately 80% of EOC but for only 50–60% of early stage tumors [13]. HE4 has a stable 4-disulfide core protein associated with the *WFDC2* gene and was first introduced as a potential biomarker for EOC in 2003 [14]. Both CA125 and HE4 have been reported to promote EOC growth and invasion although the mechanism of this action is not clearly defined [15].

In this study, we aimed to evaluate the diagnostic performance of HE4 and CA125 in blood from patients presenting with suspicious malignant ovarian cyst. We especially wanted to investigate levels of HE4 and CA125 with regard to the unifying model of type I and type II EOC.

Materials and methods

Study population

A total of 393 patients were enrolled in the study. Twenty tumors were excluded because of non-epithelial ovarian cancer (n = 4; 3 granulosa cell cancers and 1 malignant teratoma) and metastases (n = 16). The eligible study population (n = 373) comprised women with benign ovarian tumors (n = 215), borderline tumors (n = 45), and EOC (n = 113) (Table 1). Menopause status, defined as one year of amenorrhea, was checked for women between 47 and 56 years of age. Patients <47 years were considered premenopausal and women >56 years, postmenopausal. The local ethics committee at Gothenburg University approved the study protocol, and samples were collected consecutively from all patients who signed a written formed consent.

After surgery, the tumors were examined by an experienced pathologist for diagnosis, histology, grade, and stage (I–IV), according to FIGO standards. The EOC was further divided into type I and type II tumors [8]. Type I included low-grade (G1) serous, low-grade (G1) endometrioid, all clear cell, mucinous, and transitional (Brenner) carcinomas. Type II included high-grade (G2–G3) serous, high-grade (G2–G3) endometrioid, undifferentiated carcinoma, and malignant mixed mesodermal tumors (Table 2) [8].

Table 2
Epithelial ovarian cancer classification by type I (n = 42) and type II (n = 71).

Histology	Stage[a]	Grade				Total, n = 113 (100 %)
		High	Moderate	Poor	Undiff	
Mucinous	I	5	1	2		8
	II		1			1
	III	1				1
	IV	1				1
Total		7	2	2		Mucinous 11 (9.7) Type I
Clear cell	I	4		1		5
	III	1	1			2
Total		5	1	1		Clear cell 7 (6.2) Type I
Serous	I	9	7	6		22
	II	2		5		7
	III	7	8	23		38
	IV		2	3		5
Total		18 type I	17 type II	37 type II		Serous 72 (63.7)
Endometrioid	I	4	4	2		10
	II			1		1
	III	2	2	2		6
Total		6 type I	6 type II	5 type II		Endometrioid 7 (15.1)
Undifferentiated	I				2	
	III				4	Undifferentiated 6 (5.3)
Total		24 type I	23 type II	42 type II	6 type II	

[a]According to FIGO.

Sample collection and processing and biomarker analyses

Patients were consecutively and prospectively included when admitted for surgery for a clinically suspicious malignant ovarian cyst at the Department of Gynecologic Oncology, Sahlgrenska University Hospital, Gothenburg, Sweden, from 2001 to 2010. Inclusion and exclusion criteria have been specified earlier along with handling and storage of samples [11]. ELISA analyses were performed on plasma according to the manufacturer's instructions (Fujirebio Diagnostics, Inc., Gothenburg, Sweden) [11]. HE4 plasma concentrations were determined using HE4 EIA assay (Fujirebio Diagnostics) and plasma CA125 levels were measured using Architect CA 125 II (Abbott Diagnostics, USA) at Fujirebio Diagnostics. The assays were performed on coded samples.

Table 1
Type I and type II patient age, menopause status and tumor characteristics.

	Benign			Borderline			Malignant		
	Pre-M	Post-M	All (%)	Pre-M	Post-M	All (%)	Pre-M	Post-M	All (%)
Mean age (range)	41 (16–52)	66 (47–88)	60	40 (18–52)	65 (47–85)	50	44 (28–56)	65 (48–88)	61
Total 373 (393)[a] (%)	50 (23.3)	165 (76.7)	215 (57.6)	27 (60)	18 (40)	45 (12.1)	21 (18.6)	92 (81.4)	113 (30.3)
EOC type I							9 (21.4)	33 (78.6)	42 (37.2)
EOC type II							12 (17.0)	59 (83.0)	71 (62.8)
Histology									
Simple	11	36	47 (22)						
Endometrioma	5	6	11 (5)						
Hemorrhagic	2	2	4 (2)						
Stromal	2	11	13 (6)	0	1	1 (2)			
Dermoid	8	4	12 (6)	1	0	1 (2)	0		
Serous	7	69	76 (35)	17	7	24 (53)	10	62	72 (64)
Mucinous	15	37	52 (24)	8	10	18 (40)	4	7	11 (10)
Endometrioid				1	0	1 (2)	6	11	17 (15)
Clear cell							0	7	7 (6)
Undifferentiated							1	5	6 (5)

Pre-M = premenopausal; Post-M = postmenopausal.
[a] =20 tumors were excluded; 3 granulosa cell cancers, 1 malignant teratoma, and 16 metastases.

Statistical analyses

Statistical differences in protein levels between groups were evaluated using the Mann–Whitney U test or the corresponding Kruskal–Wallis one-way analysis of variance for three or more groups. Cut-off for CA125 <35 U/ml was used. For HE4 cut-off values for this study population were calculated in our prior study [11] HE4 71.8 pM premenopausal and 85 pM postmenopausal. Cases with marker levels above threshold levels were considered to have a positive result. When the markers were used in combination the test was positive if one of the markers was positive, and negative if both of the markers were negative. The predicted probabilities for each model were used to construct receiver operating characteristic (ROC) curves, and the area under the curve (AUC) values was calculated. Sensitivity and specificity were calculated for individual markers and their combinations and positive predictive value (PPV) and negative predictive value (NPV) for types I and II. The natural log of protein levels was included as independent variables in the logistic regression analysis. For all statistical comparisons a value of $p < 0.05$ was considered significant. Statistical analyses were performed in SPSS for Windows version17.0 (SPSS Inc., Chicago, IL, USA) and Stata 12.1 (Stata Corp., Texas, USA).

Results

Patient material

Of the 373 women eligible for analysis, 58% had benign tumors, 12% borderline and 30% EOC (Table 1). The malignant tumors were divided into the slow-growing type I EOC and the more aggressive type II EOC by histology and grade (Table 2). Type I included low-grade serous (n = 18; 42%), low-grade endometrioid (n = 6; 14%), all mucinous (n = 11; 26%) and all clear cell (n = 7; 17%). The type II group included high-grade serous (n = 54; 76%), high-grade endometrioid (n = 11; 15.5%), and undifferentiated carcinomas (n = 6; 8.5%). The mean age was equally distributed within the benign and EOC cohorts, type I and type II, while the mean age for the borderline tumors was 10 years younger (60% premenopausal). Most of the women with EOC (81.4%) were postmenopausal, 79% in type I and 83% in type II, and the benign cohort included 78% postmenopausal women (Table 1). The median values and range of HE4 and CA125 in all subgroups are found in the Supplement (S1).

Significantly different levels of HE4 and CA125 in the type I and type II cohorts

HE4 and CA125 significantly separated ($p < 0.001$) the type I and type II cohorts from the benign cohort, and CA125 levels were significantly ($p < 0.001$) different between the benign and borderline tumors, but not HE4 ($p = 1.0$) (Table 3) (Fig. 1A + B). Both biomarkers were also able to separate type I from type II, and borderline from type II tumors ($p < 0.001$). However, neither HE4 ($p = 0.026$) nor CA125 ($p = 1.000$) was significant when borderline tumors were compared to type I EOC. The median value in benign tumors for HE4 was 66 pM and increased to 93 pM and 354 pM in type I and type II. The increase was even more notable for CA125 where median value of CA125 ranged from 16 U/ml in benign patients to 53 U/ml in type I and 395 U/ml in type II (Table 3, Fig. 1A + B). The ROC AUC was, according to these findings, high for type II EOC (0.92 HE4; 0.93 CA125; 0.93 HE4 + CA125),

Table 3
HE4 and CA125 levels according to histology, type, stage and menopause status; significant differences, ROC AUC and sensitivity at 75% specificity in benign vs. type I and type II, and positive predictive value (PPV) and negative predictive value (NPV).

Benign	Type I EOC						Type II EOC				
	Median (range)	Median (range)	p-Value	ROC AUC (95%CI)	Sensitivity[a] (%)	PPV/NPV	Median (range)	p-Value	ROC AUC (95%CI)	Sensitivity (%)	PPV/NPV
CA125	16 (2–4632)	53 (8–3250)	<0.001	0.76 (0.68–0.85)	71.4	39.1/92.0	395 (6–14,880)	<0.001	0.93 (0.89–0.97)	93	60.7/96.6
Pre-M	23 (6–121)	40 (22–146)	0.076	0.78 (0.64–0.91)	55.6		731 (20–4232)	<0.001	0.90 (0.78–1.00)	83.3	
Post-M	14 (2–4632)	64 (8–3250)	<0.001	0.76 (0.66–0.86)	66.7		327 (6–14,880)	<0.001	0.93 (0.89–0.97)	96.6	
Early stage[b]		36 (8–2932)	<0.001	0.70 (0.60–0.81)	62.0		104 (6–2430)	<0.001	0.85 (0.76–0.94)	81.5	
Late stage[b]		194 (8–3250)	<0.001	0.9 (0.77–1.00)	92.3		564 (50–14,880)	<0.001	0.98 (0.96–0.99)	100	
HE4	66 (31–469)	93 (40–784)	<0.001	0.72 (0.63–0.81)	54.8	30.2/90.6	354 (39–7933)	<0.001	0.92 (0.87–0.96)	91.5	52.0/96.3
Pre-M	57 (31–469)	73 (46–190)	1.0	0.71 (0.52–0.90)	55.6		239 (41–1732)	<0.001	0.91 (0.77–1.00)	91.7	
Post-M	69 (34–631)	109 (0.87–0.96)	<0.001	0.73 (0.63–0.83)	60.6		412 (39–7932)	<0.001	0.91 (0.86–0.96)	91.5	
Early stage[b]		74 (40–785)	<0.001	0.66 (0.54–0.77)	45.0		132 (39–1730)	<0.001	0.81 (0.71–0.92)	81.5	
Late stage[b]		129 (66–642)	<0.001	0.86 (0.77–0.95)	76.9		474 (76–7932)	<0.001	0.98 (0.96–1.00)	97.7	
HE4 + CA125				0.79 (0.72–0.86)	61.9	31.3/95.2			0.93 (0.89–0.98)	94.4	46.5/97.2
Pre-M				0.80 (0.67–0.93)	44.4				0.92 (0.79–1.00)	83.3	
Post-M				0.79 (0.71–0.88)	66.7				0.94 (0.89–0.98)	93.2	
Early stage[b]				0.73 (0.64–0.82)	48.3				0.85 (0.75–0.95)	85.2	
Late stage[b]				0.93 (0.87–0.99)	92.3				0.99 (0.98–1.00)	100	

Early stage = I + II, late stage = III + IV; pre-M = premenopausal, post-M = postmenopausal.
[a] Sensitivity set at 75% specificity.
[b] According to FIGO staging.

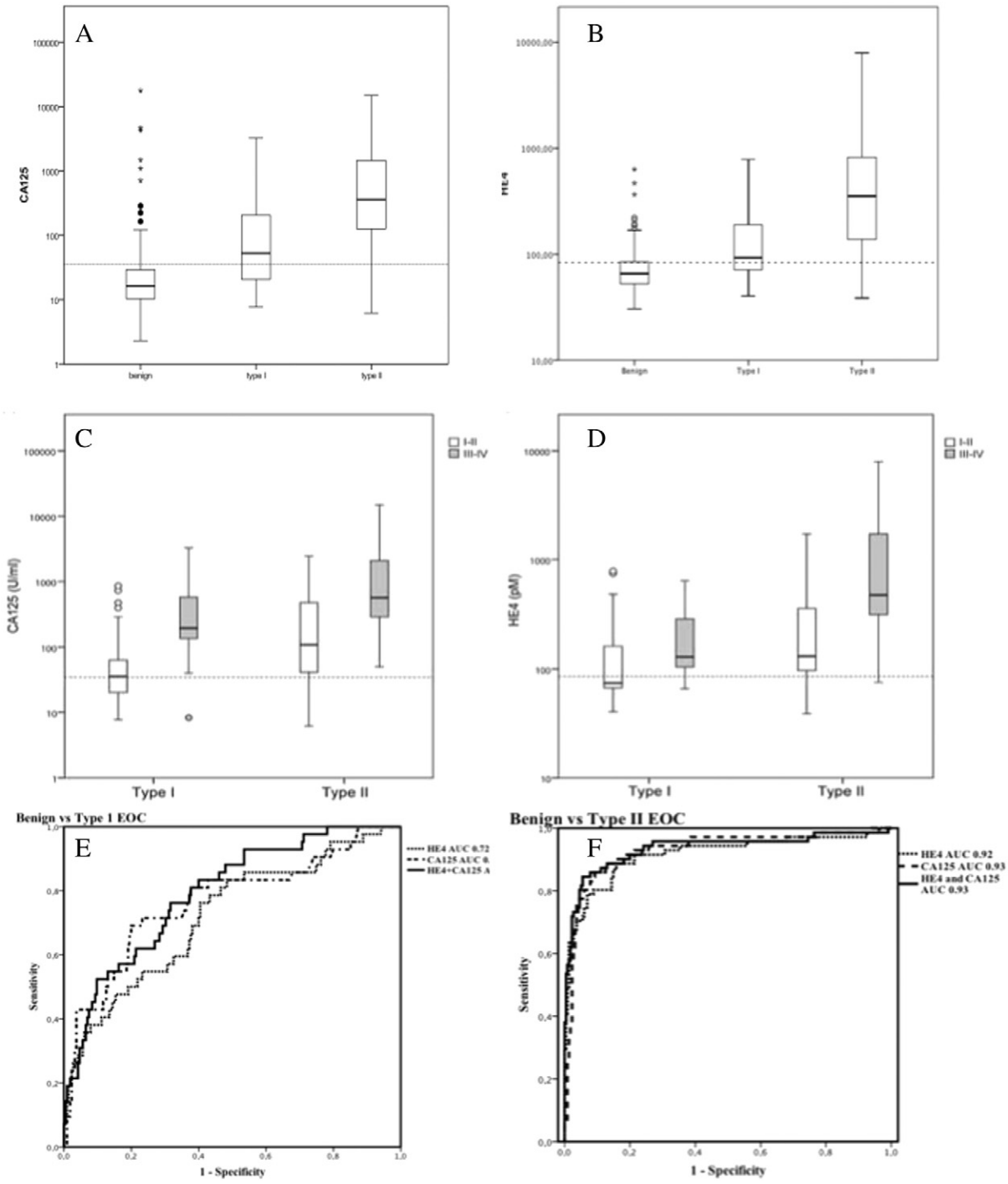

Fig. 1. Box plot for (A) CA125 levels (reference line at cut-off 35 U/ml), by benign, type I and type II EOC; (B) HE4 levels (reference line at cut-off 85 pM) by benign, type I and type II EOC; (C) CA125 levels for each type divided into early stage (FIGO I/II) and late stage (FIGO III/IV); (D) HE4 levels for each type divided into early stage (FIGO I/II) and late stage (FIGO III/IV); logarithmic scale; (E) ROC AUC for HE4, CA125, and HE4 + CA125 by benign and type I EOC; and (F) for HE4, CA125 and HE4 + CA125 by benign and type II EOC.

while the AUC was lower (0.72 HE4; 0.76 CA125; 0.79 HE4 + CA125) when benign tumors were compared to type I EOC (Fig. 1C + D). In EOC false negatives are feared. We located 13 false negatives, defined as negative by both biomarkers, all in the early stage: type I (n = 9; 5 low-grade serous, 2 mucinous, 1 endometrioid, and 1 clear cell EOC) and type II (n = 4; high-grade serous FIGO stage I). Sensitivity for HE4 and CA125 individually in type II was 91.5% and 93%, respectively, and in type I 54.8% and 71.4%, calculated at 75% specificity (Table 3). Sensitivity for type II EOC was improved (94.4%) when using the dual markers, but not in type I (61.9%), where CA125 was better used alone.

HE4 + CA125 evaluation in early and late stage type I and type II EOC

In the next step we wanted to evaluate the performance of HE4 and CA125 according to FIGO stages. Type I was divided into early stage (FIGO I + II; n = 29) 69% and late stage (FIGO III + IV; n = 13) 31%, and type II early stage (n = 27) 38% and late stage (n = 44) 62%, and

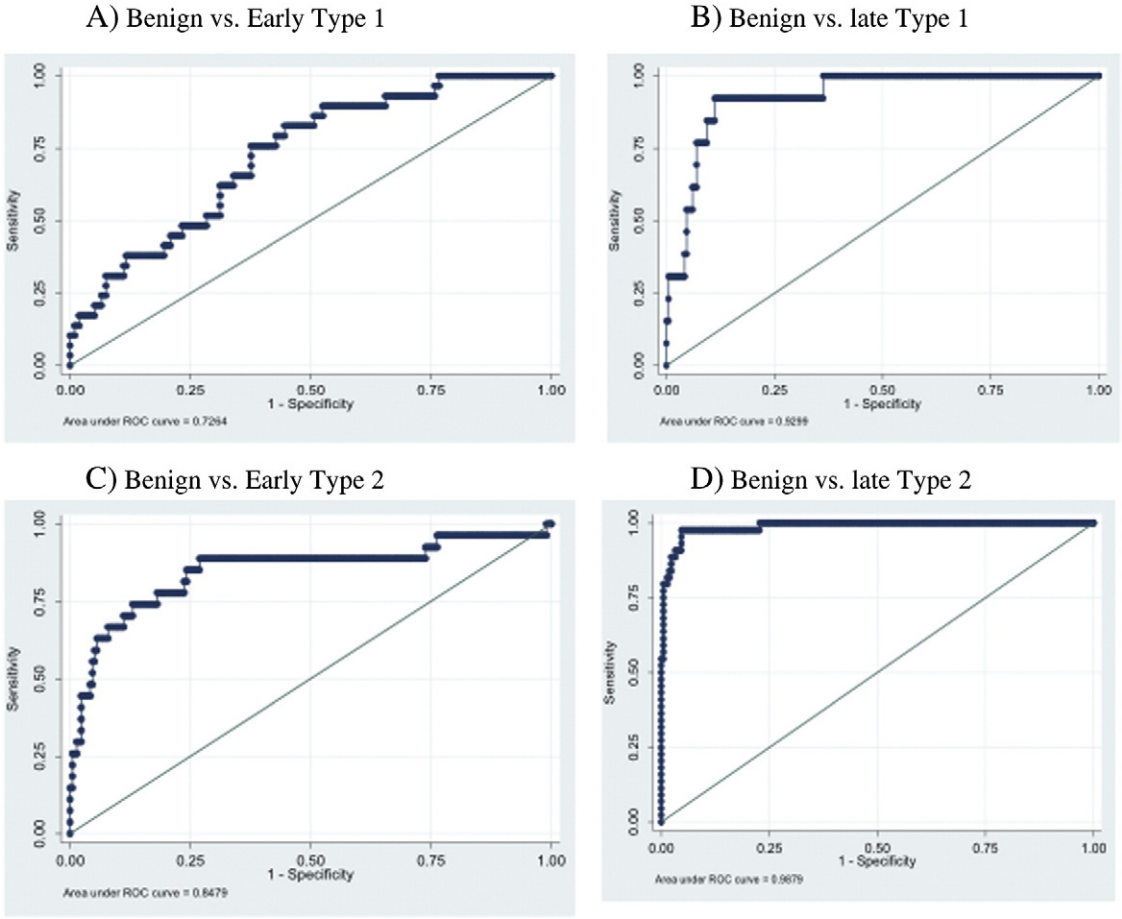

Fig. 2. (A) ROC AUC for HE4 + CA125 by benign and early (I–II) type I EOC and (B) by benign and late (III–IV) type I; (C) ROC AUC for HE4 + CA125 by benign and early type II EOC and (D) by benign and late type II.

compared to the benign cohort. Statistically significant ($p < 0.001$) differences were noted in all comparisons (Table 3, Fig. 2A–B). The median values for HE4 in early stage type I and type II EOC were 74 pM and 132 pM and in late stage were 129 pM and 474 pM respectively. The median values for CA125 in early stage type I and type II EOC were 36 U/ml and 104 U/ml and in late stage were 194 U/ml and 564 U/ml, respectively. The ROC AUC was impressively high (0.99) for HE4 +CA125 when comparing benign to late stage type II EOC and was 0.93 in type I. However, ROC AUC was just 0.85, for the clinically more sought early stage diagnostic of type II EOC and only 0.73 for type I (Fig. 2C–D). With respect to the small group size, sensitivity in early and late stage type II was 85.2% and 100% and in early and late stage type I was 48.3% and 92.3%, calculated at 75% specificity.

HE4 + CA125 evaluation in pre- and postmenopausal type I and type II EOC

We then hypothesized that the dual biomarker combination would perform even worse in premenopausal than in women in postmenopause, within type I and II compared to the benign cohort (Fig. 3). Statistically significant ($p < 0.001$) differences were found between all groups except for the premenopausal type I EOC ($n = 9$) vs. benign (Table 3). The combination of HE4 and CA125 resulted in the best ROC AUC estimates, with better value in type II, but without differences within each type: benign vs. type I pre- and postmenopausal women (0.80 and 0.79) and benign vs. type II pre- and postmenopausal (0.92 and 0.94).

Evaluation of HE4 and CA125 levels according to EOC histological subtype

The division of EOC into the proposed type I and type II tumors is based on new evidence of genetic changes in EOC histologic subgroups. As the above data shows, the dual markers could not diagnose type I EOC. Further subgrouping and analysis of the EOC type I into mucinous, endometrioid, serous type I and serous type II was performed. Low HE4 median value was detected in mucinous carcinomas (88 pM) and in clear cell carcinomas (129 pM), while HE4 for endometrioid and serous carcinomas was higher (171 pM and 322 pM) and the highest for undifferentiated tumors (629 pM). The median CA125 value was as well as for HE4 lowest in mucinous carcinomas (36 U/ml), with increasing values in endometrioid (132 U/ml), clear cell (194 U/ml), and serous carcinomas (297 U/ml) and highest in undifferentiated carcinomas (1304 U/ml) (details are found in S1). Both biomarkers were significantly different (HE4 $p = 0.0045$, CA125 $p = 0.0002$) in comparing benign serous tumors to serous type I and serous type II EOC ($p = 0.0001$). Significant differences within serous borderline tumors, type I and type II were estimated. CA125 was significantly different between these tumors, but not HE4 ($p = 0.0001$ and 0.569). However, HE4, but not CA125, was significantly increased ($p = 0.003$ and $p = 1.0$) in serous type I compared to serous borderline tumors. Neither HE4 nor CA125 showed significance comparing mucinous adenoma and borderline tumors and mucinous EOC (S1). As expected, HE4, but not CA125, was significant ($p = 0.0019$ and $p = 0.1380$) when comparing benign endometrioma from endometrioid EOC, more often negative in endometrioma.

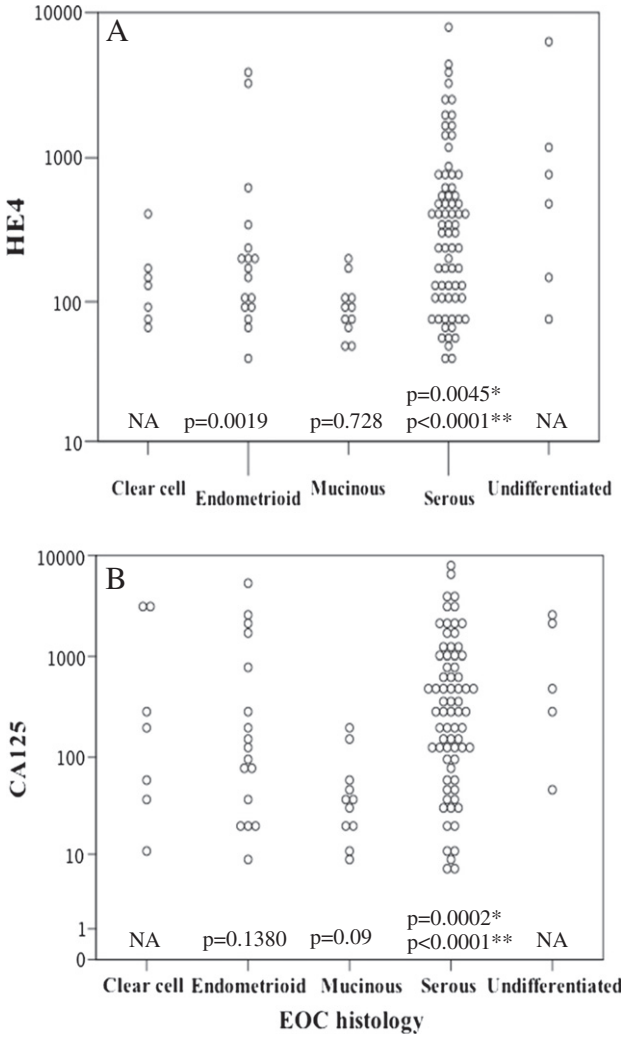

Fig. 3. (A) Scatter plot for HE4 in different histology of EOC and (B) for CA125; logarithmic scale.

Discussion

It has been suggested that EOC, which traditionally is subgrouped according to histology, should be subgrouped with regard to molecular genetic changes into slow-growing type I and aggressive type II EOC [8]. The rarity of finding early stage high-grade serous tumors (type II) has made it difficult to study pre-malignant and early stage lesions. Detecting these most threatening tumors is challenging [16,17]. In this study, we aimed to evaluate the diagnostic performance of the biomarkers HE4 and CA125 alone or combined in type I and type II EOC. We used a cohort of 373 patient blood samples that were consecutively and prospectively collected from women scheduled for surgery for a malignant suspicious cystic ovarian mass [11] and can conclude that HE4 and CA125 are highly representative markers for type II EOC. The combination of HE4 and CA125 resulted in the best diagnostic power with ROC AUC 0.93 and NPV 97.2 for type II EOC, and impressively high AUC 0.99 for late stage type II EOC in comparison to the benign cohort. But as mentioned above aggressive early stage serous EOC is in need to be correctly diagnosed, still in our study we missed four out of six. In addition, none of the markers alone or in combination had a good diagnostic ability for all type I EOC. Lu et al. tested fourteen serologic markers to discriminate type I and II EOC in a similar setting; CA125 had the greatest power of the tested markers with AUC 0.93 in type II and AUC 0.89 in type I. This is about the same as for CA125 in this study (HE4 was not included in the Lu et al. study) [18]. To our knowledge, HE4 has not earlier been evaluated by dividing EOC into type I and type II. Other reports and our previous reports have mainly studied the diagnostic performance of HE4 and CA125 in groups of all EOC compared to benign cohorts and have found less diagnostic power (AUC < 0.90) [11,12]. We believe that this is due to the poor performance of the dual biomarkers in type I EOC found in this study. We suggest that research is needed with a focus on new histology-specific markers to correctly diagnose each subgroup.

The suggested division into type I and type II on a 2-tier grading system was done based on biologic evidence that indicated that low-grade and high-grade EOC developed via different pathways. Both high-grade serous and endometrioid EOC are genetically unstable, contain P53 mutation, and behave aggressively. In contrast, low-grade tumors have a relatively stable genome and are more often confined to the ovaries at diagnosis [3,7]. This is a simplified model, putting the focus on the most aggressive EOC, the majority of all ovarian cancers. Recent studies have suggested that an even more individualized subgrouping might be needed [1,19]. In our study, the median age among EOC type I and II was the same. Age, reflected by dividing type I and type II into pre- and postmenopausal groups, did not influence ROC AUC within each group [14,20]. The high number (78%) of postmenopausal women with benign disease and the overall low number of premenopausal women may explain the relatively high age of the benign cohort. The performance of the biomarker combination was only influenced by stage within each type.

The ultimate goal to increase survival in EOC is finding early stage lesions regardless of type. In this study, early stage EOC comprised 50% of the malignant cohort (26% type I and 24% type II), which is more than expected [7,19]. The large numbers with early EOC may be due to one of the inclusion criteria: the presence of cystic ovarian pelvic mass and not just any pelvic mass. The dual biomarkers tested in this study were inferior in type I EOC diagnostics but seem to be an asset in the diagnosis of type II EOC. Tumors in the type I subgroup are generally of a larger size, more often localized in the pelvis, and therefore, more easily detected at an earlier stage with conventional techniques (such as clinical examination including gynecological bimanual palpation, transvaginal and abdominal ultrasound and computed tomography scanning) than type II EOC, which might not have visible early lesion [21].

It has been argued that the aggressive type II EOC, which mostly consists of tumors with serous histology, would benefit most from being detected at early stages [18]. In our previous study, CA125 showed better diagnostic ability than HE4 (AUC HE4 0.72 and CA125 0.76) when comparing benign to stage I EOC (n = 47/113). In contrast, HE4 could diagnose stage I EOC better than CA125 in studies by Moore (n = 13/67) and Van Gorp (n = 43/131) (AUC HE4 0.77/CA125 0.70 and AUC HE4 0.77/CA125 0.75). The three studies differed in number of included patients and the patients had different characteristics. This study was the only one that included women with cystic tumors, while Van Gorp's and Moore's cohorts had a higher percentage of premenopausal women and late stage EOC [11,12,22]. In addition, Van Gorp had 29% endometriosis in the benign group which, together with the young cohort, could theoretically increase the diagnostic capacity of HE4. However, the largest published and still ongoing Danish study present data from 1218 patients referred to a tertiary center because of a pelvic mass with high and equal capacity for both markers in diagnosing early stage tumors (n = 64/252 EOC) AUC HE4 0.86 and CA125 0.85 [23].

HE4 levels in blood and gene expression vary between different malignant histological groups with the highest values for tumors representing type II tumors [24,25]. We found significant variations in HE4 and CA125 levels within different tumors and histotypes, and as expected, the highest levels were in the tumors representing type II (S1). HE4 could significantly separate endometrioma from endometrioid EOC but none of the markers could separate benign and borderline mucinous tumors from mucinous EOC. This is in keeping with several recent papers, which mainly investigated EOC in late stages [22,24,26]. Great diagnostic power for serum CA125 (AUC 0.99) and HE4 (AUC 0.98) for

serous EOC was found along with equally bad diagnostic performance in mucinous tumors [24,26]. Drapkin et al. demonstrated 93% expression of HE4 in high-grade serous, 100% in high-grade endometrioid, and 50% in clear cell, but no staining in mucinous when studying 92 late stage EOC [25].

Type I EOC is a group of rare and individually different tumors that need attention. Mucinous tumors can still be challenging to differentiate using vaginal ultrasound; even during an expert pathologic examination, metastases from the gastrointestinal tract of mucinous histology are often mistaken for ovarian cancer [1,27]. Though belonging to type I EOC, clear cell and mucinous tumors are quite aggressive, particularly at late stages with an even higher mortality than type II [19,28]. Finding early markers that are specific for all histology subgroups is a future challenge. Achieving a better understanding of the pathogenesis, molecular biology, and behavior of the EOC is crucial to move forward the process of improving early diagnosis and survival for patients with EOC.

Conclusion

Using HE4 and CA125 we can more accurately diagnose the aggressive type II, than the type I EOC. The diagnostic safety for the dual markers HE4 and CA125 is not acceptable in early stage type I EOC. Our results support the hypothesis that EOC should be looked upon as several different diseases. Finding early markers that are specific for all histologic subgroups will be our future challenge.

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.ygyno.2013.07.094.

Conflict of interest statement

The manufacturer of HE4 EIA assay (Fujirebio Diagnostics) in Gothenburg, Sweden performed all the analyses on plasma for both HE4 and CA125, and any data regarding the patients or tumors was blinded. The authors declare that there are no conflicts of interest.

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