Clin Chem Lab Med 2011;49(12):2081–2088 © 2011 by Walter de Gruyter • Berlin • Boston. DOI 10.1515/CCLM.2011.709

The diagnostic accuracy of two human epididymis protein 4 (HE4) testing systems in combination with CA125 in the differential diagnosis of ovarian masses

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Abstract

Background: Cancer antigen 125 (CA125) is the best known single tumor marker for ovarian cancer (OC). We investigated whether the additional information of the human epididymis protein 4 (HE4) improves diagnostic accuracy.
Methods: We retrospectively analyzed preoperative sera of 109 healthy women, 285 patients with benign ovarian masses (cystadenoma: n=78, leimyoma: n=66, endometriosis: n=52, functional ovarian cysts: n=79, other: n=10), 16 low malignant potential (LMP) ovarian tumors and 125 OC (stage I: 22, II: 15, III: 78, IV: 10). CA125 was analyzed using the ARCHITECT system, HE4 using the ARCHITECT(a) system and EIA(e) technology additionally.
Results: The lowest concentrations of CA125 and HE4 were observed in healthy individuals, followed by patients with benign adnexal masses and patients with LMP tumors and OC. The area under the curve (AUC) for the differential diagnosis of adnexal masses of CA125 alone was not significantly different to HE4 alone in premenopausal (CA125: 86.7, HE4(a): 82.6, HE4(e): 81.6% $p>0.05$) but significantly different in postmenopausal [CA125: 93.4 vs. HE4(a): 88.3 $p=0.023$ and vs. HE4(e): 87.8% $p=0.012$] patients. For stage I OC, HE4 as a single marker was superior to CA125, which was the best single marker in stage II-IV. The combination of CA125 and HE4 using risk of malignancy algorithm (ROMA) gained the highest sensitivity at 95% specificity for the differential diagnosis of adnexal masses [CA125: 70.9, HE4(a): 67.4, HE4(e): 66.0, ROMA(a): 76.6 and ROMA(e): 74.5%], especially in stage I OC [CA125: 27.3, HE4(a): 40.9, HE4(e): 40.9, ROMA(a): 45.5 and ROMA(e): 45.5%].
Conclusions: CA125 is still the best single marker in the diagnosis of OC. HE4 alone and even more the combined analysis of CA125 and HE4 using ROMA improve the diagnostic accuracy of adnexal masses, especially in early OC.

Keywords: CA125; human epididymis protein 4 (HE4); ovarian cancer; risk of malignancy algorithm (ROMA); tumor marker.

Introduction

Ovarian cancer (OC) is the leading cause of death among gynecologic malignancies. Primary treatment includes operative cytoreduction and subsequent platinum-based combined chemotherapy. Reported response rates to standard primary treatment range around 80%, but 60%–70% of patients with OC relapse or die within 5 years after diagnosis (1–3). Due to the lack of diagnostic tools for early detection of OC, the vast majority of patients are detected at a progressed stage of disease (4). Only 25%–30% of all OC patients are diagnosed at an early stage and have better survival rates (5, 6). Persistent ovarian masses at subsequent vaginal sonographies are difficult to handle in clinical routines. To rule out a malignant mass, operative exploration is frequently recommended to the patient. Although most lesions turn out to be benign, some are OCs (7). The proportion of malignant ovarian tumors is higher in postmenopausal in comparison to premenopausal women.

Besides vaginal sonography, tumor marker values aid in deciding which patients to operate on. Cancer antigen 125 (CA125) as a single marker has been shown to be elevated in the majority of patients with OC. Moreover, its level is known to be related to stage and histological type. Still, the diagnosis and differential diagnosis remains difficult as 20% of all OC patients present with negative CA125 and a high percentage of patients with benign diseases show increased tumor marker values (8, 9). So far, the combination of CA125 and the human epididymis protein (HE4) measured by enzyme immunometric assay (EIA) technology (Fujirebio Diagnostics AB, Sweden) has been described to be of additive value in the differential diagnosis of pelvic masses (10, 11). The aim of this study was to assess whether the recently developed automated test for HE4 on the ARCHITECT system (Abbott Diagnostics, USA) leads to comparable clinical results in combination with CA125.

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Received April 18, 2011; accepted August 8, 2011; previously published online September 16, 2011

Materials and methods

Patients

Women diagnosed and treated for low malignant potential (LMP) tumors of the ovary or OC between 1985 and 2008 were included in the study population. In the retrospective evaluation, tumor marker levels were analyzed with reference to patient characteristics and clinical data including menopausal status, histology or tumor stage. Healthy women and patients treated for benign ovarian masses served as controls. The study was approved by the Local Ethical Committee. Informed consent was obtained from all patients participating in this study.

Serum analysis

All serum samples had been obtained preoperatively at primary diagnosis and had been stored at –80°C. CA125 and HE4 were analyzed in parallel using the ARCHITECT system [Abbott, ARCHITECT, Abbott Park, IL, USA (a)] and using the EIA technology (Fujirebio Diagnostics AB, Sweden, (e)] according to the manufacturers' instructions.

The ARCHITECT assay for HE4 and CA125 are both two-step immunoassays. In the first step, sample and OC 125 coated or 2H5 anti-HE4 coated paramagnetic microparticles were combined. OC 125 defined antigen sample or HE4 antigen then bind to the OC 125 coated or anti-HE4 coated microparticles. In the second step, M11 acridinium-labeled conjugate was added after washing in the CA125 assay and 3D8 anti-HE4 acridinium labeled conjugate in the HE4 assay. Chemilumniscent reaction was measured by relative light units and directly reflects CA125 and HE4 concentrations in the serum samples.

The HE4 EIA is a solid-phase, non-competitive immunoassay using the same 2H5 and 3D8 antibodies as described for the ARCHITECT assay. In short, samples are incubated with biotinylated anti-HE4 monoclonal antibody (MAb) 2H5 in streptavidin coated microstrips. After washing incubation with HRP, labeled anti-HE4 MAb 3D8 was performed. Color intensity was measured to calculate HE4 serum concentration.

Menopausal stage was defined according to follicle-stimulating hormone level concentrations measured. To assess the diagnostic value of HE4 and CA125 in combination, the final ROMA was used as described elsewhere (11):

Premenopausal patients:

Predictive Index (PI) $= -12.0 + 2.38 \times \mathrm{LN}[\mathrm{HE4}] + 0.0626 \times \mathrm{LN}[\mathrm{CA125}]$

Postmenopausal patients:

Predictive Index (PI) $= -8.09 + 1.04 \times \mathrm{LN}[\mathrm{HE4}] + 0.732 \times \mathrm{LN}[\mathrm{CA125}]$

Risk of malignancy $= \exp(\mathrm{PI})/[1 + \exp(\mathrm{PI})] \times 100$.

Histology

All patients were operated on by experienced gynecological surgeons. Tumor typing and staging were performed by experienced gynecological pathologists according to the criteria of the International Federation of Gynaecologists and Obstetricians (FIGO) and the International Union against Cancer (IUCC). Histology, FIGO stage and tumor grade according to the World Health Organization (WHO) were recorded.

Statistics

Statistical analysis was performed using SAS V9.2 (SAS Institute Inc., Cary NC, USA). Tumor marker results are given as median, range and percentile. Differences between groups were evaluated using the non-parametric Wilcoxon test (e.g., histological subtype) or the non-parametric Jonckheere-Terpstra test (e.g., tumor stage). The Passing and Bablok regression was performed to test for the equality of measurements of the two different analytical HE4 technologies (12). Moreover, the Spearman correlation coefficient was calculated for the values obtained with the HE4 EIA and HE4 ARCHITECT systems.

The AUCs were determined to reflect the relationship between sensitivity and specificity for single tumor markers or tumor marker combinations. To compare AUCs for statistical significance, p-values were calculated (13). Sensitivities were also calculated at set specificities of 75%, 90% and 95% for each marker and marker combinations. p-Values of <0.05 were regarded as statistically significant.

Results

Patient characteristics

We retrospectively analyzed preoperative sera of 109 healthy women, 285 patients with benign ovarian masses, 16 LMP tumors of the ovary and 125 OC. Patient's characteristics including median age, menopausal status, histology, stage and grade are shown in detail in Table 1.

Tumor marker values

The Passing and Bablok regression analysis indicated comparable results for the two HE4 technologies (all values: P/B regression $\mathrm{HE4(a)} = 1.024 \times \mathrm{HE4(e)} + 6.030$; $n = 646$). There was a good correlation between HE4 EIA and HE4 ARCHITECT measurement (Spearman: $r = 0.91$) (Figure 1).

The lowest median concentrations of CA125 and HE4 were observed in healthy individuals, followed by patients with benign disorders and patients presenting with LMP tumors. The highest marker concentrations were observed in OC patients. For each sub-group, the two HE4 technologies showed similar values within the group (Table 2).

For the histological type, CA125 showed the highest serum concentrations in serous OC patients. In general, HE4 measured by ARCHITECT and EIA showed comparable results within each histological group of OC patients. The highest median HE4 release was found for serous OCs, followed by endometrioid tumors. Significantly different marker levels between histological subtypes were observed between serous and mucinous OC in CA125 ($p = 0.0011$) and HE4 ARCHITECT ($p = 0.0029$) as well as HE4 EIA ($p = 0.0026$) and also comparing serous to endometrioid OC in CA125 levels ($p = 0.0063$) (Table 2).

With regard to tumor stage, the lowest median tumor marker values were found in patients with stage I OC. Significant different tumor marker levels with regard to tumor stage were observed for CA125 ($p < 0.001$), HE4 ARCHITECT ($p < 0.0098$) and HE4 EIA ($p < 0.0089$). Again, the serum testing for HE4 with the ARCHITECT or EIA technology showed comparable results within each group of patients (Table 3).

ROC curves were calculated for the comparison of patients with benign ovarian masses vs. OC and LMP tumor

Table 1 Patients' characteristics for healthy women, patients with benign ovarian masses, LMP or OC including median age, menopausal status, histology, stage and grade.

Patients	Number (n)	Median age (range)	Menopausal status (n)	Histology (n)	Stage (FIGO)	Grade
Healthy women	109	38.4 (21.5–80.0)	Premenopausal: 81 Postmenopausal: 20 NA: 8			
Benign ovarian mass	285	44.6 (18.5–87.3)	Premenopausal: 160 Postmenopausal: 125	Cystadenoma: 78 Leiomyoma: 66 Endometriosis: 52 Functional ovarian cyst: 79 Inflamm. adnexal disease: 3 Teratoma: 7		
LMP	16	59.7 (23.6–88.3)	Premenopausal: 3 Postmenopausal: 13	Endometrioid: 1 Mucinous: 7 Serous: 8	I: 15 II: 0 III: 1	G2: 2 NA: 14
OC	125	62.9 (22.7–88.2)	Premenopausal: 27 Postmenopausal: 98	Endometroid: 12 Mucinous: 8 Serous: 84 Other: 21	I: 22 II: 15 III: 78 IV: 10	G1: 8 G2: 30 G3: 68 NA: 19

patients for the single marker HE4(a), HE4(e) and CA125 as well as ROMA using either HE4(a) or HE4(e) (Table 4). The combined analysis of CA125 and HE4 (ROMA), either with the ARCHITECT or EIA, was significantly superior to both HE4 tests alone [HE4(e) vs. ROMA(e) $p=0.008$, HE4(a) vs. ROMA(a) $p=0.013$]. No significant differences were observed between the ROC curves for CA125 in comparison to HE4 or ROMA using both HE4 technologies [CA125 vs. HE4(e) or vs. HE4(a) $p>0.05$, CA125 vs. ROMA(e) or vs. ROMA(a) $p>0.05$]. Moreover, there were no significant differences between both HE4 testing systems and between both ROMA results [HE4(e) vs. HE4(a) $p>0.05$, ROMA(e) vs. ROMA(a) $p>0.05$] (Figure 2).

At a set specificity of 95%, sensitivities were lowest for all single markers or the marker combinations at premenopausal status. When comparing single markers and ROMA in premenopausal patients, the AUCs of CA125, HE4(e) and HE4(a) and both ROMA were not significantly different ($p>0.05$ each).

However, AUCs for both ROMA and both HE4 were significantly different in comparison to CA125 alone in postmenopausal patients [CA125 vs. HE4(a) $p=0.023$, CA125 vs. HE4(e) $p=0.012$, CA125 vs. ROMA(a) $p=0.001$, CA125 vs. ROMA(e) $p=0.001$ (Table 4)].

Table 5 shows the results for the comparison of patients with a benign ovarian mass vs. patients with OC for different tumor stages. Sensitivities were calculated at set specificities of 75%, 90% and 95%, respectively. When raising specificity, the highest loss in sensitivity is generally found in early stage disease. However, the combination of both ROMA gained the highest sensitivity at 95% specificity for stage I OC patients, followed by HE4 alone and CA125 alone. Only at stage I OC, CA125 seems to be inferior to both HE4 and both ROMA (Table 5).

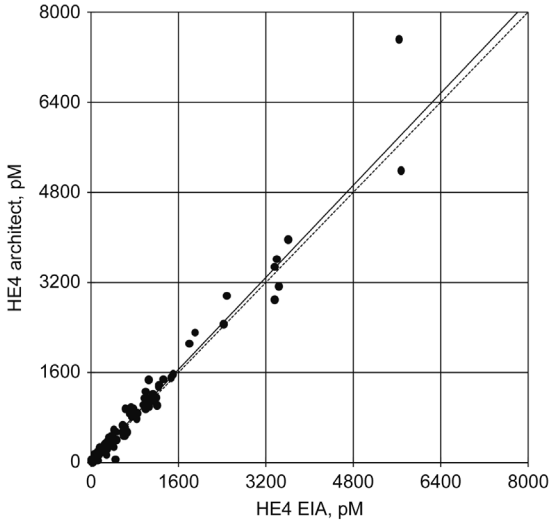

Figure 1 The Passing and Bablok regression for the two HE4 technologies (all values: P/B regression $Y=1.024\times X+6.030$; $n=646$; Spearman: $r=0.91$).

Discussion

OC is often detected at progressed stage and ranks as the fifth most common cause of death in women (14). So far, data for OC screening with vaginal sonography, clinical examination and tumor marker evaluation are not satisfactory (15, 16). Moreover, the interpretation of tumor marker elevation remains difficult for the known variation in tumor marker release due to inflammation, endometriosis, liver disease, ovulation or menopausal status (17, 18), which we could also observe in our patient population.

Tumor marker CA125

There have been numerous studies describing preoperative CA125 in OC patients and few studies for ovarian borderline

Table 2 CA125 and HE4 (ARCHITECT and EIA) concentrations for healthy women, patients with benign ovarian masses, patients diagnosed with LMP tumor or OC showing median, range, 5th and 95th percentile (Pctl). In addition, CA125 and HE4 (ARCHITECT and EIA) concentrations are shown in detail for the histological subtypes. Significantly different results were observed between serous and mucinous OC in CA125 ($p = 0.0011$), HE4 ARCHITECT ($p = 0.0029$) and HE4 EIA ($p = 0.0026$) and comparing serous to endometrioid OC in CA125 ($p = 0.0063$).

Group	n	Marker	Unit	Median	Range	5th Pctl	95th Pctl
Healthy women	109	CA 125 ARCHITECT	U/mL	13.5	4.0–49.7	7.4	30.0
		HE4 ARCHITECT	pmol	40.4	4.5–111	15.2	72.0
		HE4 EIA	pmol	32.5	2.5–444	2.5	77.2
Benign ovarian mass	285	CA 125 ARCHITECT	U/mL	19.6	4.0–276	8.4	124
		HE4 ARCHITECT	pmol	40.3	18.8–1178	25.0	94.6
		HE4 EIA	pmol	35.3	2.5–1120	2.5	96.7
LMP	16	CA 125 ARCHITECT	U/mL	34.7	18.1–385	18.1	335
		HE4 ARCHITECT	pmol	52.1	28.2–399	28.2	399
		HE4 EIA	pmol	43.7	13.6–441	13.6	441
OC	125	CA 125 ARCHITECT	U/mL	391	12.5–35813	28.3	7075
All		HE4 ARCHITECT	pmol	242	29.0–7507	37.7	2954
		HE4 EIA	pmol	246	9.6–5669	33.8	3357
Serous	84	CA 125 ARCHITECT	U/mL	656.5	16.2–35813	39.4	3321
		HE4 ARCHITECT	pmol	386.0	31.6–7507	54.2	3124
		HE4 EIA	pmol	364.0	17.2–5669	40.8	3398
Mucinous	8	CA 125 ARCHITECT	U/mL	126.5	29.2–285	29.2	285
		HE4 ARCHITECT	pmol	74.1	35.4–147	35.4	447
		HE4 EIA	pmol	75.6	33.8–335	33.8	335
Endometrioid	12	CA 125 ARCHITECT	U/mL	70.3	21.3–1749	21.3	1749
		HE4 ARCHITECT	pmol	165.5	39.7–3469	39.7	3469
		HE4 EIA	pmol	146.0	33.7–3357	33.7	3357
Other	21	CA 125 ARCHITECT	U/mL	211.0	12.5–13804	20.3	1564
		HE4 ARCHITECT	pmol	161.0	29.0–2301	33.0	1134
		HE4 EIA	pmol	146.0	9.6–1901	31.7	1202

patients (19–21). OC patients, but also ovarian borderline tumor patients are known to present with elevated CA125 in most cases (21, 22). Our data confirm that serum CA125 in OC patients differ in median from healthy controls, patients with benign gynecologic disease and patients with ovarian borderline tumors. Patients with OC generally show the high-

Table 3 CA125 and HE4 (ARCHITECT and EIA) concentrations in OC patients, detailed for tumor stage I-IV, showing median, range, 5th and 95th percentile (Pctl). Significant differences between stages were observed for CA125 (two sided $p < 0.001$), HE4 ARCHITECT (two sided $p < 0.0098$) and HE4 EIA (two sided $p < 0.0089$).

Group	n	Marker	Unit	Median	Range	5th Pctl	95th Pctl
All OC	125	CA125	U/mL	391	12.5–35813	28.3	7075
		ARCHITECT	pmol	242	29.0–7507	37.7	2954
		HE4 ARCHITECT	pmol	246	9.6–5669	33.8	3357
		HE4 EIA					
FIGO I	22	CA125	U/mL	38.2	12.5–1564	16.2	1459
		ARCHITECT	pmol	66.0	29.0–2301	33.0	1134
		HE4 ARCHITECT	pmol	71.3	9.6–1901	31.7	1100
		HE4 EIA					
FIGO II	15	CA125	U/mL	1519	34.6–5855	34.6	5855
		ARCHITECT	pmol	538	53.2–7507	53.2	7507
		HE4 ARCHITECT	pmol	624	43.8–5633	43.8	5633
		HE4 EIA					
FIGO III	78	CA125	U/mL	505	28.5–35813	56.2	9227
		ARCHITECT	pmol	340	33.2–5184	50.5	3124
		HE4 ARCHITECT	pmol	317	32.1–5669	35.8	3398
		HE4 EIA					
FIGO IV	10	CA125	U/mL	559	28.3–8323	28.3	8323
		ARCHITECT	pmol	300	31.6–1114	31.6	1114
		HE4 ARCHITECT	pmol	341	17.2–1076	17.2	1076
		HE4 EIA					

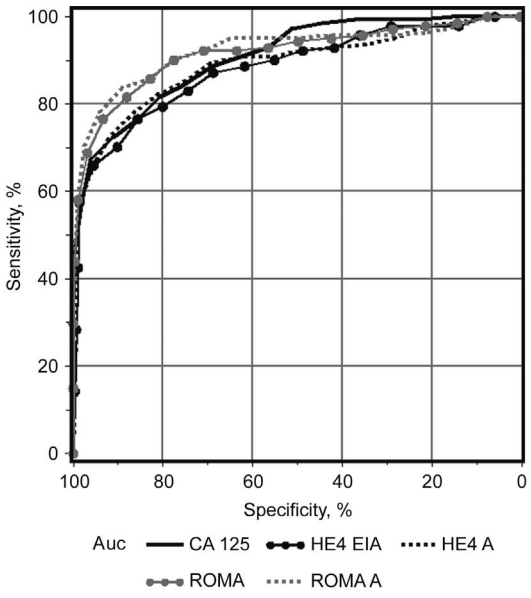

Figure 2 ROC curves for CA125, HE4 [EIA and ARCHITECT(a)] and ROMA using either the HE4 EIA or ARCHITECT(a) technology.
Diagnostic accuracy is calculated for patients with benign ovarian mass vs. all OC patients and patients with LMP tumors.

est levels of CA125, healthy women the lowest levels. Moreover, patients with progressed OC present with higher tumor marker values, which was also described in literature (23). Median tumor marker values differ in patients with various histological subtypes of OC. Our data show serous OC patients to have the highest CA125 concentrations in median, which agrees with the data of other centers (23, 24).

Tumor marker HE4

CA125 is the most commonly used tumor marker in the diagnosis and differential diagnosis of pelvic masses (10, 11). New tumor markers like HE4 are gaining importance. Drapkin et al. showed that HE4 is distributed in a region of the cytoplasm with a perinuclear pattern reminiscent of the endoplasmic reticulum and the Golgi apparatus (24). The HE4 gene encodes a protease inhibitor with a role in protective immunity and is primarily expressed in the reproductive tract and upper airways (24, 25). Moreover, the HE4 gene product is N-glycosylated and secreted into the extracellular environment of cancer cells, and elevated serum levels can be measured in these patients. Some studies analyzed HE4 in gynecological cancer patients (26). We concentrated on OC and ovarian borderline tumor patients with appropriate control groups of gynecological patients and healthy women and compared the results of two different HE4 measurement systems. Tumor marker values for HE4 were lowest in stage I disease with both technologies used. The data agrees with the results published by Montagnana et al. (10), who also found a stage dependent HE4 release in OC. Interestingly, HE4 was more frequently expressed in early stage disease when compared to CA125. Especially at very high specificity, HE4 alone achieves 40.9% sensitivity with both HE4 techniques. In comparison to single HE4 values, CA125 alone only has a sensitivity of 27.3% and is therefore inferior to HE4 testing. Especially at early stage disease, HE4 seems to improve the identification of OC patients. Our results are in accordance with the data published by Havrilesky et al. (27).

Similar to CA125, HE4 shows the highest tumor marker values for serous OC patients, which is also in agreement with data in literature (10). Interestingly, HE4 levels are also relatively high in patients with endometrioid tumors. We recently found HE4 to be less frequently elevated in benign gynecological disease (data not presented in detail for different diseases here) like endometriosis, where CA125 can attain relatively high serum values. Still, there are diseases in which HE4 also lacks specificity, like inflammation or renal failure (28). Moreover, recent data describe a significant variation of HE4 release in healthy women under the age of 35, which depends on the female hormonal cycle. Therefore, Anastasi et al. point out that it is necessary to properly interpret HE4 data with regard to the female hormonal cycle (29).

Marker combination

Various studies investigated the use of tumor marker combinations in OC patients (11, 27, 30–37). The combination of CA125 and HE4 seems to be favorable in most studies for the differential diagnosis of these patients (31, 34). Moore et al. used the dual marker combination of HE4 and CA125 in ROMA to classify women into high and low risk groups (11).

In this study, we assessed whether the automated test for HE4 with the ARCHITECT system and the manual HE4 EIA lead to comparable clinical results in combination with CA125.

The results achieved with the dual marker combination and information on the patient's menopausal status, as performed in ROMA, are comparable to the published result of the most commonly used risk of malignancy index (RMI) (38–40), RMI utilizes a combination of serum CA125 values, pelvic sonography and the menopausal status (41). In Jacobs et al. a sensitivity of 95.1% is noted at 76.5% specificity (42). In a recently published study, Moore et al. compared the RMI with ROMA (43). At a set specificity of 75%, ROMA had a sensitivity of 94.3% and RMI had a sensitivity of 84.6% for distinguishing benign findings from EOC (43). Further analysis for patients with early stage disease showed a sensitivity of 85.3% for ROMA compared to 64.7% for RMI. The authors therefore concluded that the dual marker algorithm utilizing HE4 and CA125 achieves a higher sensitivity than RMI in the diagnosis of OC (43).

A recently published study by Montagnana et al. concludes ROMA to be excellent in the detection of OC of postmenopausal but not premenopausal women (30). This finding goes along with our results as we observed AUCs of 83.1% with ROMA using the ARCHITECT and 82.0% using the EIA technology in premenopausal women in comparison to 93.9% (ARCHITECT) and 93.2% (EIA) in postmenopau-

Table 4 Sensitivity at 95% specificity and AUC values with lower confidence limits (LCL) and upper confidence limits (UCL) in the differentiation of benign and malignant gynecological masses for CA125, HE4 ARCHITECT, HE4 EIA and ROMA using the HE4 ARCHITECT and HE4 EIA system. Results are also shown in detail by menopausal status.

Patients	Marker	Sensitivity at 95% specificity	AUC % (LCL–UCL)
All patients:	CA 125 ARCHITECT	70.9%	91.1 (88.2–94.0)
benign ovarian mass	HE4 ARCHITECT	67.4%	89.0 (85.4–92.7)
(n = 285) vs. OC + LMP (n = 141)	HE4 EIA	66.0%	88.2 (84.5–91.9)
	ROMA (HE4 ARCHITECT)	76.6%	92.4 (89.3–95.6)
	ROMA (HE4 EIA)	74.5%	91.8 (88.6–95.0)
Premenopausal patients:	CA 125 ARCHITECT	60.0%	86.7 (78.8–94.5)
benign ovarian mass	HE4 ARCHITECT	66.7%	82.6 (72.1–93.5)
(n = 160) vs. OC + LMP (n = 30)	HE4 EIA	56.7%	81.6 (71.1–92.0)
	ROMA (HE4 ARCHITECT)	66.6%	83.1 (72.6–93.7)
	ROMA (HE4 EIA)	56.7%	82.0 (71.7–92.3)
postmenopausal patients:	CA 125 ARCHITECT	73.0%	93.4 (90.6–96.3)
benign ovarian mass	HE4 ARCHITECT	66.8%	88.3 (84.0–92.7)
(n = 125) vs. OC + LMP (n = 111)	HE4 EIA	64.9%	87.8 (83.4–92.2)
	ROMA (HE4 ARCHITECT)	72.1%	93.9 (91.1–96.7)
	ROMA HE4 EIA)	71.2%	93.2 (90.2–96.3)

sal women. This variability should always be taken into account when ROMA score results are compared (30).

Still, results on CA125, HE4 and ROMA are partly controversial. Ruggeri et al. found HE4 to be more specific and accurate than CA125 (34), whereas Van Gorg et al. concluded HE4 and ROMA not to increase the detection of ovarian cancer (37). Jacob et al. analyzed HE4 and CA125 with or without ROMA and RMI and concluded not to see a benefit from combining HE4 and CA125 as tumor markers in a clinical setting (36).

We observed the highest benefit for HE4 and ROMA in OC patients and early stage disease. This finding was also described by others (11, 36).

Altogether, the differences in CA125, HE4 and ROMA results may be attributable to various numbers of patients, tumor stages, menopausal status or histologic subtypes in the ovarian cancer patient study groups and also control groups.

Strengths of this study are the large patient and control groups, the persistent high standard of surgery by gynecologic oncologists at a specialized academic institution and

Table 5 Tumor marker sensitivity at set specificity of 75%, 90% and 95% for CA125, HE4 ARCHITECT, HE4 EIA, ROMA using the HE4 ARCHITECT and ROMA using the HE4 EIA, detailed for tumor stage I–IV.

Benign ovarian mass (n = 285) vs.	Marker	Sensitivity at 75% specificity	Sensitivity at 90% specificity	Sensitivity at 95% specificity
OC	CA125 ARCHITECT	50.0%	27.3%	27.3%
FIGO I	HE4 ARCHITECT	68.2%	45.5%	40.9%
(n = 22)	HE4 EIA	63.6%	50.0%	40.9%
	ROMA (HE4 ARCHITECT)	86.4%	59.1%	45.5%
	ROMA (HE4 EIA)	81.8%	59.1%	45.5%
OC	CA 125 ARCHITECT	93.3%	86.7%	86.7%
FIGO II	HE4 ARCHITECT	100%	80.0%	80.0%
(n = 15)	HE4 EIA	100%	80.0%	80.0%
	ROMA (HE4 ARCHITECT)	93.3%	86.7%	86.7%
	ROMA (HE4 EIA)	93.3%	86.7%	80.0%
OC	CA125 ARCHITECT	98.7%	92.3%	89.7%
FIGO III	HE4 ARCHITECT	94.9%	88.5%	83.3%
(n = 78)	HE4 EIA	92.3%	85.9%	80.8%
	ROMA (HE4 ARCHITECT)	98.7%	96.2%	92.3%
	ROMA (HE4 EIA)	98.7%	93.6%	89.7%
OC	CA125 ARCHITECT	90.0%	80.0%	80.0%
FIGO IV	HE4 ARCHITECT	80.0%	80.0%	70.0%
(n = 10)	HE4 EIA	80.0%	70.0%	70.0%
	ROMA (HE4 ARCHITECT)	80.0%	80.0%	80.0%
	ROMA (HE4 EIA)	80.0%	80.0%	80.0%

the consistent histopathologic review by expert gynecologic oncology pathologists.

Conclusions

In the differential diagnosis of OC patients, CA125 still represents the best single tumor marker. The combined analysis of CA125 and HE4, using either the ARCHITECT or EIA system, improves the diagnostic accuracy in the distinction of ovarian tumors, especially in early OC. Both HE4 testing methods can be used in clinical routines since the HE4 ARCHITECT shows comparable results with the HE4 EIA technology.

Acknowledgments

We thank Dr. Barry Dowell and Sabrina Chan (Abbott Diagnostics, USA) und Dr. Olle Nilsson (Fujirebio Diagnostics, Sweden) for their support with reagents and equipment and for contributing their scientific know how in data evaluation and discussion.

Conflict of interest statement

Authors' conflict of interest disclosure: The authors stated that there are no conflicts of interest regarding the publication of this article.
Research funding: None declared.
Employment or leadership: None declared.
Honorarium: None declared.

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