Identification of high-risk patients by human epididymis protein 4 levels during follow-up of ovarian cancer

KARINA DAHL STEFFENSEN[1,2], MARIANNE WALDSTRØM[3], IVAN BRANDSLUND[4], BENTE LUND[5], SARAH MEJER SØRENSEN[6], MAX PETZOLD[7] and ANDERS JAKOBSEN[1,2]

[1]Department of Oncology, Vejle Hospital, DK-7100 Vejle; [2]Institute of Regional Health Research, University of Southern Denmark, DK-5000 Odense; Departments of [3]Pathology and [4]Clinical Biochemistry, Vejle Hospital, DK-7100 Vejle; [5]Department of Oncology, Aalborg University Hospital, DK-9100 Aalborg; [6]Faculty of Health Sciences, University of Copenhagen, DK-2200 Copenhagen, Denmark; [7]Centre for Applied Biostatistics, Sahlgrenska Academy, University of Gothenburg, S-405 30 Gothenburg, Sweden

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Abstract. The majority of ovarian cancer patients with advanced disease at diagnosis will relapse following primary treatment, with a dismal prognosis. Monitoring the levels of serum markers in patients under follow-up may be essential for the early detection of relapse, and for distinguishing high-risk patients from those with less aggressive disease. The aim of the present study was to investigate the possible predictive value of human epididymis protein 4 (HE4) and carbohydrate antigen 125 (CA125) in relation to recurrence of epithelial ovarian cancer by measuring the two markers during follow-up subsequent to surgery and adjuvant first-line carboplatin/paclitaxel chemotherapy. Serum HE4 and CA125 were analyzed in 88 epithelial ovarian cancer patients at the end of treatment and consecutively during follow-up. The patients were divided into a high-risk and a low-risk group based on having an increase in HE4 and CA125 levels above or below 50% during follow-up, relative to the baseline (end-of-treatment) level. Disease recurrence was detected in 55 patients during follow-up. Patients with an increase in HE4 of >50% at 3- and 6-month follow-up compared to the end-of-treatment sample had significantly poorer progression-free survival (PFS) [hazard ratio (HR), 2.82 (95% CI, 0.91-8.79; P=0.0052) and HR, 7.71 (95% CI, 3.03-19.58; P<0.0001), respectively]. The corresponding 3- and 6-month biomarker assessments for increased CA125 levels (>50%) showed HRs of 1.86 (95% CI, 0.90-3.80; P=0.0512) and 2.55 (95% CI, 1.39-4.68; P=0.0011), respectively. Multivariate analysis confirmed HE4 as a predictor of short PFS, with an HR of 8.23 (95% CI, 3.28-20.9; P<0.0001) at 6-month follow-up. The increase of CA125 was not a significant prognostic factor in multivariate analysis for PFS. In conclusion, HE4 appears to be a sensitive marker of recurrence and instrumental in risk assessment during the first 6 months of follow-up.

Correspondence to: Dr Karina Dahl Steffensen, Department of Oncology, Vejle Hospital, Kabbeltoft 25, DK-7100 Vejle, Denmark
E-mail: karina.dahl.steffensen@rsyd.dk

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Introduction

The majority of patients with advanced ovarian cancer will experience disease recurrence within a few years from the time of diagnosis (1). Due to the nature of the disease, with its location in the small pelvis and spread in the form of diffuse carcinosis, there are no reliable methods of detecting early recurrence using ultrasound, computed tomography (CT) or magnetic resonance imaging (2,3). Furthermore, it is well known that serum levels of carbohydrate antigen 125 (CA125) have a considerable lead-time before clinically detectable recurrence (4,5).

Currently, the clinical consequence of rising CA125 levels remains an issue of great debate, particularly as to whether re-treatment should be initiated based only on biochemical CA125 recurrence. Rustin *et al* (6) demonstrated that survival time was not improved when treatment was based on biochemical recurrence alone. However, it should be noted that this study investigated only women who experienced normalization of CA125 during their first-line treatment. Furthermore, the study has been criticized for its very diverse relapse treatment, including different chemotherapy regimens, which may not have been in accordance with the current standard. Also, none of the patients were offered secondary cytoreductive surgery that may have led to improved survival; this issue is currently under intense discussion in the scientific community, and the results of the ongoing randomized DESKTOP III (7) study are eagerly awaited to clarify whether surgery for relapse will improve the outcome for patients with recurrent disease. It may be highly relevant to practice active monitoring of women after the end of treatment to detect recurrence as early as possible, particularly if the DESKTOP III study demonstrates a survival benefit. Furthermore, a retrospective study by Fleming *et al* (8) indicated that each week delay of treatment following the first CA125 elevation in recurrent ovarian cancer correlated with a 3% increased chance of suboptimal resection at secondary

cytoreductive surgery, and therefore CA125 surveillance increased optimal resectability at secondary cytoreductive surgery. Thus, the current situation calls for better methods for early detection of recurrence with the perspective of curatively intended surgical and/or non-surgical treatment. CA125 is insufficient for a number of reasons, including the fact that it is not always elevated in patients with mucinous tumors (9).

Human epididymis protein 4 (HE4) is a relatively new biomarker approved by the United States Food and Drug Administration (FDA) for monitoring of patients with epithelial ovarian cancer. HE4 is encoded by the *WFDC2* gene located on chromosome 20q12-13.1 (10) and belongs to the family of whey-acidic four-disulfide core proteins with suspected trypsin-inhibitor properties (11). However, the biological role of HE4 has not yet been identified (12). HE4 is upregulated in ovarian cancer compared to other types of carcinomas and benign ovarian tumors (13,14). Recent studies have identified HE4 as a complementary marker for ovarian cancer that can be elevated in some cases where CA125 is not (15-21); however, its potential value in the early detection of recurrence has not been elucidated.

The aim of the present study was to explore the clinical value of serial measurements of HE4 and CA125 during follow-up for the early detection of recurrence, and the additive value of combining the two markers.

Materials and methods

Study population. The current study included patients with ovarian cancer who had completed first-line combination chemotherapy with paclitaxel (175 mg/m^2 intravenously) and carboplatin (AUC=5) every 3 weeks at two Danish Hospitals (Aalborg University Hospital, Aalborg, Denmark; and Vejle Hospital, Vejle, Demark) and attended follow-up according to national guidelines. Patients with a serum sample drawn at the end of chemotherapy and ≥2 post-chemotherapy blood samples were included in the study.

All patients were entered as part of a translational research protocol, with peripheral venous blood samples drawn at the end of chemotherapy and at every scheduled follow-up visit: Every 3 months for the first two years, every 6 months for the third year, and once a year for the fourth and fifth years. Clinical data were recorded in detailed case report forms. Detailed patient characteristics have been given elsewhere (22).

The study was carried out in compliance with the Helsinki II Declaration (23) and all patients signed an informed consent form. The Danish Biomedical Research Ethics Committee and the Danish Data Protection Agency approved the study according to Danish law. Recurrence of disease was defined according to the Gynecological Cancer Intergroup CA125 criteria (24,25) and/or radiological confirmation of tumor recurrence, whichever occurred first. Biochemical recurrence detected by CA125 required CT confirmation to verify the diagnosis of recurrence.

Serum CA125 assay. The quantitative levels of serum CA125 were determined using the commercially available CanAg CA125 Enzyme Immunometric Assay (EIA) kit (cat. no.,400-10; Fujirebio Diagnostics AB, Gothenburg, Sweden) with inter- and intra-assay coefficients of variation (CV) of ≤10% and a sensitivity of 1.5 IU/ml. The assay is based on a direct sandwich technique using two mouse monoclonal antibodies, Ov197 and Ov185, directed against two independent epitopes of the protein core of the CA125 antigen. The analysis was performed in 25 μl of serum and followed the manufacturer's protocol.

Serum HE4 assay. HE4 serum levels were determined by the enzyme-linked immunosorbent assay technique using a commercially available FDA-approved kit (HE4 EIA kit, cat. no., 404-10; Fujirebio Diagnostics AB). The analysis used 25 μl serum; the analytical steps were conducted according to the manufacturer's instructions and have been described in further detail in a previous publication from our group (21).

HE4 control 1 and 2 were used for validation of each assay series. The lyophilized controls contained HE4 antigen in a human serum matrix and a non-azide antimicrobial preservative included in the kit. The mean values of control duplicates and the duplicate replicates of calibrators A-F were within the specified ranges provided by the manufacturer for all runs. The total CV in the present analysis was between 3.3 and 8.8% in the high and low range of HE4 levels, respectively.

Statistical analyses. A validated HE4 threshold for monitoring during follow-up has not been established, and the current study aimed to investigate changes in HE4 and CA125 during follow-up compared to their 'baseline' level at the end of adjuvant chemotherapy treatment. At 3- and 6-month follow-up examinations, the HE4 and CA125 levels were compared to the baseline end-of-treatment (EOT) sample, and patients were divided into groups based on having an increase above or below/equal to 50%. From this dichotomous classification, a univariate Kaplan-Meier log-rank analysis was performed to assess the association with progression-free survival (PFS).

Multivariate analysis (Cox regression) was conducted for the identification of independent factors predicting PFS.

The study also aimed to investigate whether the EOT sample drawn at the end of chemotherapy (corresponding to the beginning of follow-up) was able to predict disease recurrence at this very early time point. It was decided *a priori* that the data would be analyzed at a set sensitivity of 90% since the aim was to investigate whether the markers were sensitive enough to detect relapse from a serum sample taken just before the follow-up. A sensitivity of 90% for detecting recurrence was achieved when the threshold was 41 pmol/l for HE4, and 1 U/ml for CA125. The latter, which is the lowest detectable level, was not meaningful in the analysis of specificity, positive predictive value (PPV) or negative predictive value (NPV) for CA125.

For the analysis of HE4 and CA125 thresholds, simple and multiple regression analyses were used. Statistical analyses were performed with NCSS software (version 2007; NCSS, Kaysville, UT, USA; www.ncss.com) and STATA 13.1 (College Station, TX, USA). The Mann-Whitney U test was used for the comparison of medians. $P<0.05$ was considered to indicate statistically significant differences.

Results

Patient characteristics. From May 2006 through August 2011, a total of 283 consecutive patients were enrolled in the

Table I. Patient characteristics (n=88).

Clinicopathological parameter	Value
Age, years	
Median	64.0
Range	28-77
FIGO stage, n (%)	
I	22 (25.0)
II	7 (8.0)
III	44 (50.0)
IV	15 (17.0)
Grade[a], n (%)	
1	11 (16.9)
2	25 (38.5)
3	29 (44.6)
Histological type, n (%)	
Serous	65 (73.9)
Mucinous	4 (4.5)
Endometrioid	9 (10.2)
Clear cell	6 (6.8)
Other[b]	4 (4.5)
Residual tumor, n (%)	
0 cm	56 (63.6)
<1 cm	11 (12.5)
≥1 cm	21 (23.9)

[a]n=65 (23 patients not graded, e.g. clear cell, carcinosarcoma or biopsy only); [b]carcinosarcoma, undifferentiated, transitiocellular, mixed or carcinoma not further classified. FIGO, International Federation of Gynecology and Obstetrics.

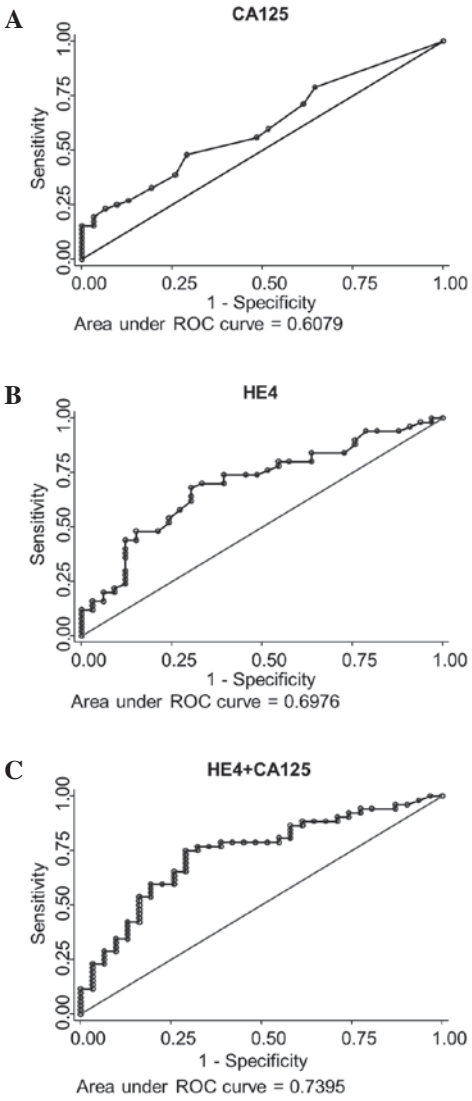

Figure 1. ROC curves for women with ovarian cancer with relapse as the endpoint. Risk stratification was performed using blood sample drawn at the end of first line adjuvant treatment prior to follow-up. (A) CA125, (B) HE4 and (C) combined HE4 and CA125 at the end of treatment for cases (relapse) vs. patients with no relapse during follow-up. ROC, receiver operating characteristic; HE4, human epididymis protein 4; CA125, carbohydrate antigen 125.

translational research protocol, and all had serum samples drawn during chemotherapy. In July 2008, the protocol was amended and approved for collection of blood samples during follow-up, and 88 patients were identified as having a serum EOT sample and ≥2 sequential samples collected during the follow-up period, according to the inclusion criteria. The median follow-up time for patients still alive (n=52) was 47 months (range, 26-86 months). Of the 88 patients, 55 were diagnosed with recurrence and 38 patients died during follow-up. More than 97.7% had ≥3 serial serum samples, and 72.7% had ≥4 serial samples (maximum, 12). The median time between collection of the EOT sample and the first follow-up sample at 3 months was 93 days (31-147 days) and the median time to the sample drawn at 6-month follow-up was 187.5 days (68-266 days). In total, 547 serum samples were analyzed: 83 EOT samples and 464 samples during the subsequent follow-up period. Patient characteristics are presented in Table I.

Prediction of relapse from EOT samples by CA125, HE4 and combined CA125/HE4 levels. The median CA125 serum level at the end of first-line chemotherapy treatment (prior to the initiation of follow-up) was 4 U/ml (95% CI, 1-5 U/ml; range 1-14 U/ml) for patients without relapse and 5 U/ml (95% CI, 3-7 U/ml; range 1-116 U/ml) for patients with relapse (P=0.0985, Mann-Whitney U test).

The median HE4 serum level at the end of first-line chemotherapy treatment (prior to the initiation of follow-up) was 51 pmol/l (95% CI, 46-59 pmol/l; range, 15-127 pmol/l) for patients without relapse and 67 pmol/l (95% CI, 60-81 pmol/l; range, 31-229 pmol/l) for patients with relapse (P=0.0013, Mann-Whitney U test).

Fig. 1 illustrates the receiver operating characteristic curve analysis for samples collected at the end of first-line treatment prior to follow-up (EOT sample), and prediction of recurrence with relapse (PFS) as the endpoint.

HE4 values at the end of first-line treatment classified 70 patients (84.3%) as being in the high risk of relapse group, and 13 (15.7%) into the low-risk group, with a sensitivity of 90.0% (95% CI, 79.0-96.8%), a specificity of 25.8% (95% CI, 11.9-44.6%), a PPV of 67.1% (95% CI, 54.9-77.9%) and an NPV of 61.5% (95% CI, 31.6-86.1%) (data not shown).

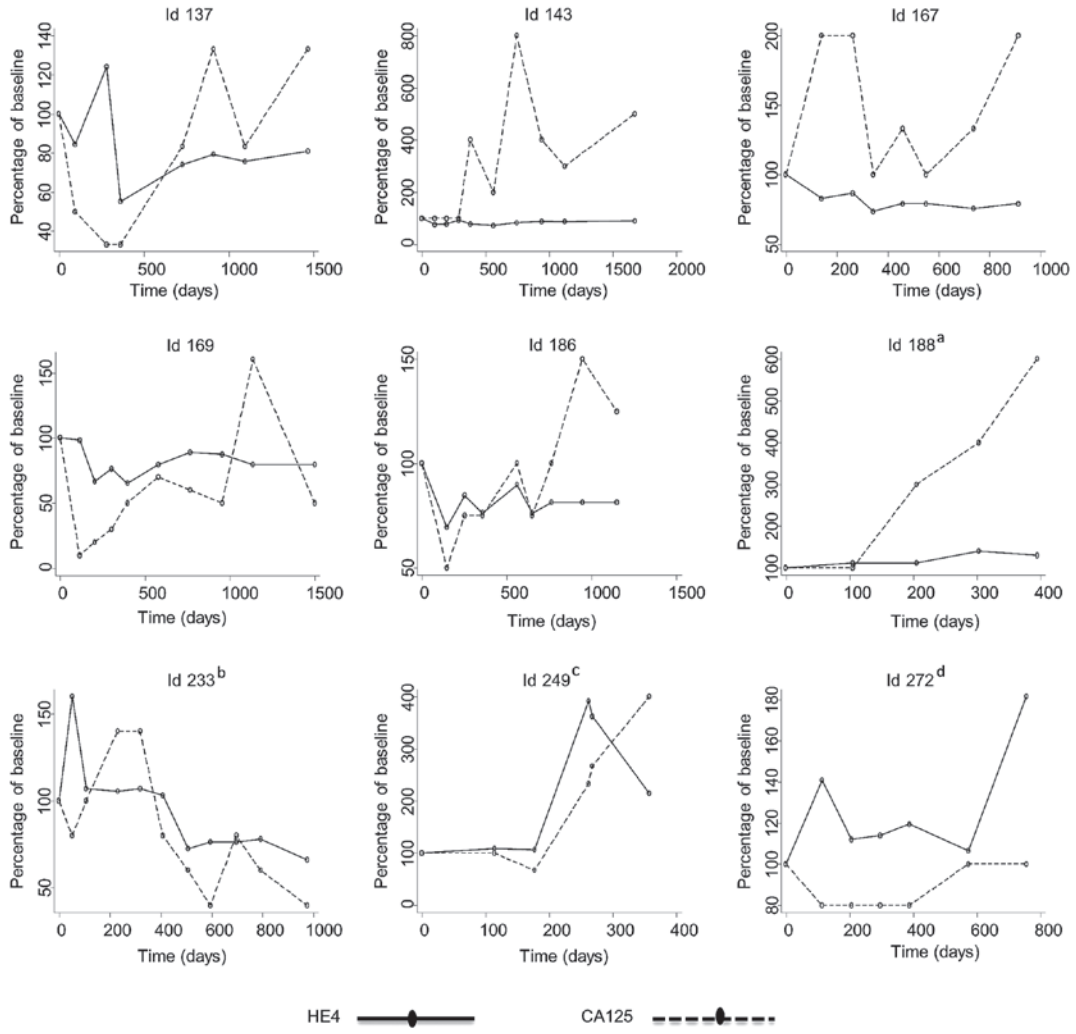

Figure 2. Graphs showing the development (in percentage compared to the end-of-treatment sample) of CA125 and HE4 levels during follow-up for selected patients without disease relapse during follow-up. [a]Id 188 with HE4 levels of 100-150 pmol/l during follow-up was diagnosed with a metastasizing pancreatic tumor at 16-month follow-up. [b]Id 233 only had increased HE4 shortly after the end of chemotherapy treatment, which dropped to within the normal range during follow-up. [c]Id 249 was diagnosed with a new primary tumor (a lung adenocarcinoma) at the time of increasing HE4; HE4 increased from 102 to 376 pmol/l in 3 months just prior to the lung cancer diagnosis. [d]Id 272 had HE4 >150 pmol/l without clinical signs of relapse. HE4, human epididymis protein 4; CA125, carbohydrate antigen 125.

When combining HE4 and CA125, 69 patients (83.1%) were in the high-risk and 14 (16.9%) in the low-risk group, resulting in a sensitivity of 90.0% (95% CI, 79.0-96.8%), a specificity of 29% (95% CI, 14.2-48.0%), a PPV of 68.1% (95% CI, 55.8-78.8%) and an NPV of 64.3% (95% CI, 35.1-87.2%) (data not shown).

Prediction of relapse during follow-up. Fig. 2 (patients without relapse) and Fig. 3 (patients with relapse) illustrate selected cases during the follow-up process, in which the first serum sample is the EOT sample and subsequent samples were obtained during the follow-up visits. Analysis of HE4 and CA125 levels at 3 months after EOT (Fig. 4A and B) revealed that an increase of ≥50% (high-risk patients) relative to the level at EOT is associated with a significant worsening of PFS. In particular, a HE4 increase >50% was correlated with significant worsening of PFS time [hazard ratio (HR), 2.82; 95% CI, 0.91-8.79; P=0.0052, log-rank test]. Additionally, increased CA125 (>50%) was associated with poorer PFS time (HR, 1.86; 95% CI, 0.90-3.80; P=0.0487, log-rank test).

The median PFS was 25.1 months (95% CI, 17.7-56.8) if HE4 did not increase >50% compared to the EOT sample, while it was 11.1 months (95% CI, 11.0-12.4) for an increase >50% at the 3-month follow-up.

For CA125 at 3 months, the median PFS was 28.7 months (95% CI, 17.4-56.8) if stable, and 13.4 months (95% CI, 11.5-24.9) if increased >50%.

The impact of increased HE4 became more clear after 6 months of follow-up (HR, 7.71; 95% CI, 3.03-19.58; P<0.0001, log-rank test) (Fig. 4D and E), with a median PFS time of 56.8 months (95% CI, 28.7-63.2) if stable, compared with 11.4 months (95% CI, 10.8-12.1) when HE4 increased >50%. The corresponding median PFS values were 56.8 months (95% CI, 28.7-63.2) vs. 14.2 months (95% CI, 12.0-7.4) for CA125 (HR, 2.55; 95% CI, 1.39-4.68, P=0.0011, log-rank test).

Combining the two markers and classifying the patients into a high-risk group if both markers had increased >50% revealed similar results: P<0.0001 with a median PFS of 25.1 months (95% CI, 17.7-56.8) if both markers were stable, and 11.5 months (95% CI, 9.2-11.6) if both markers increased

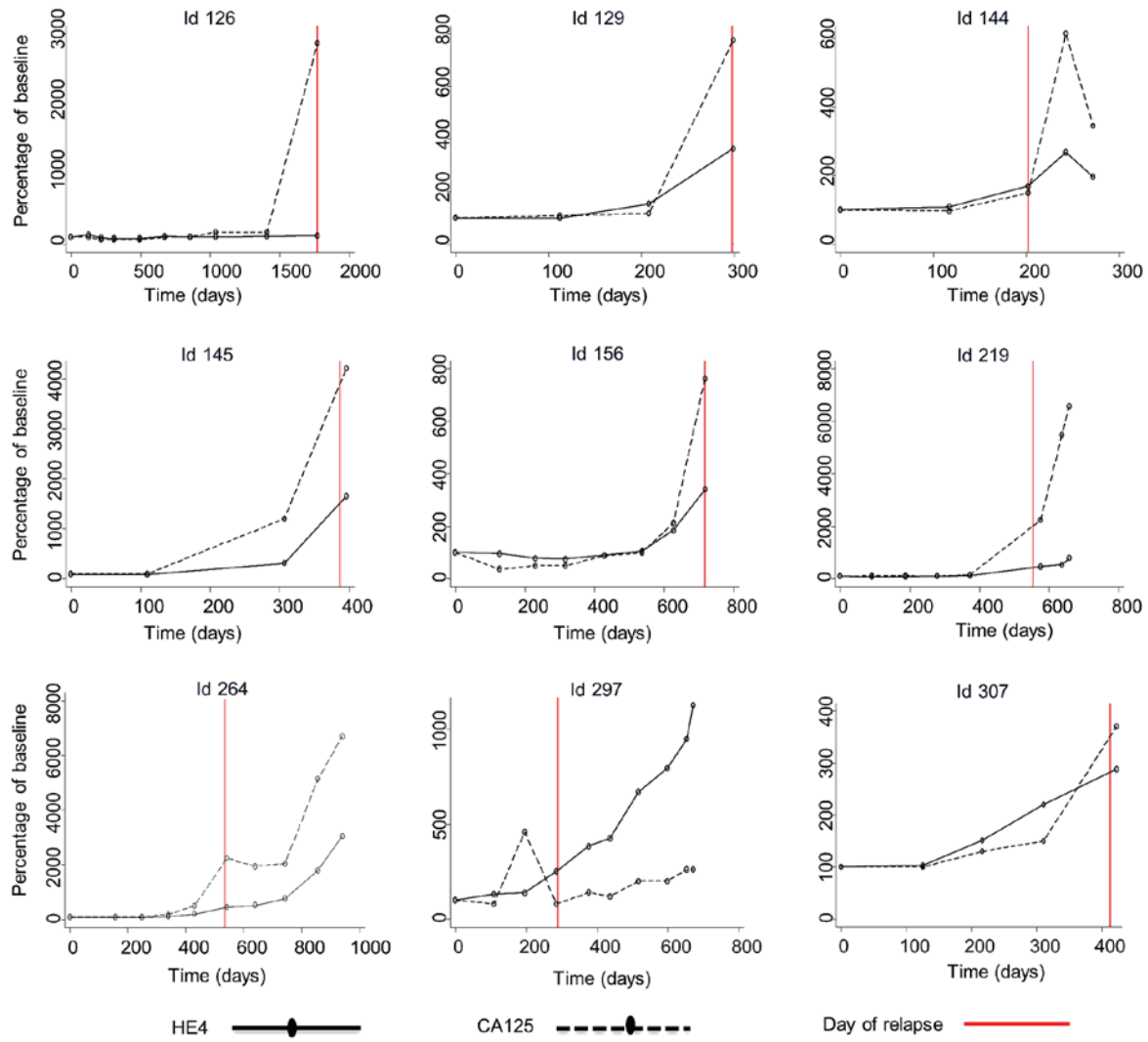

Figure 3. Graphs showing the development (in percentage compared to the end-of-treatment sample) of CA125 and HE4 levels during follow-up for selected patients with progressive disease/relapse during follow-up. The vertical line illustrates the time of relapse. HE4, human epididymis protein 4; CA125, carbohydrate antigen 125.

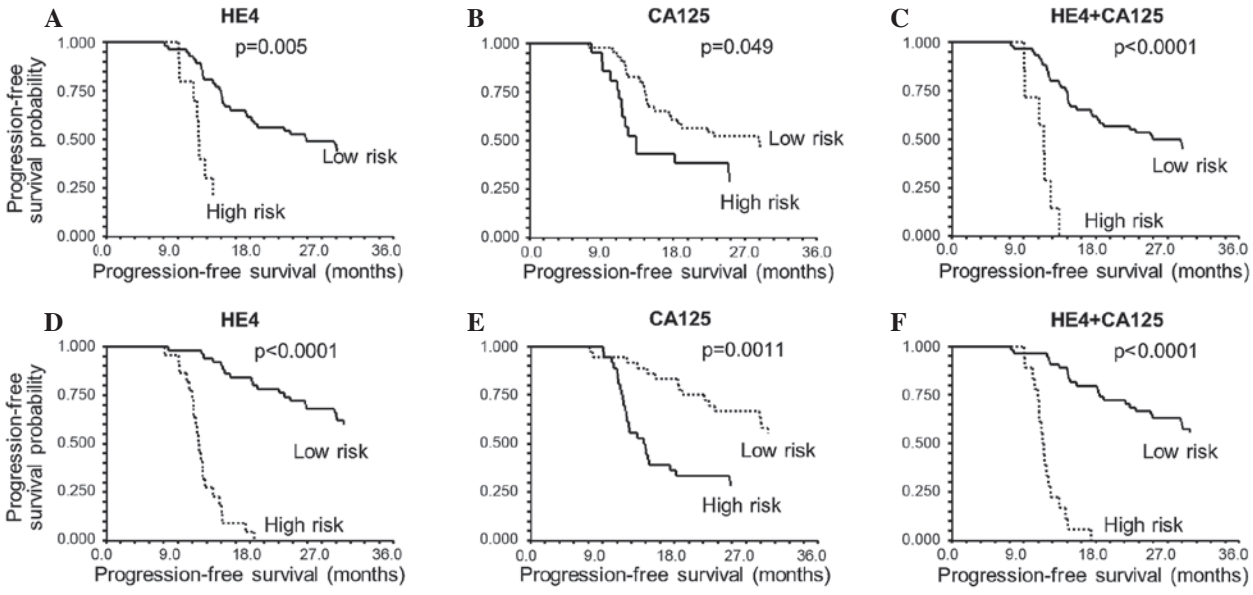

Figure 4. Kaplan-Meier curves for progression-free survival analysis for samples analyzed at (A-C) 3-month and (D-F) 6-month follow-up. Low risk indicates no increase in marker compared to the end-of-treatment sample; high risk indicates an increase in marker of ≥50% compared to the end-of-treatment sample. HE4, human epididymis protein 4; CA125, carbohydrate antigen 125.

Table II. Multivariate progression-free survival analysis for 3 and 6 months of follow-up.

	3-month follow-up			6-month follow-up		
Variable	HR	95% CI	P-value	HR	95% CI	P-value
Age	0.97	0.94-1.00	0.081	0.96	0.93-1.00	0.034
FIGO stage						
I/II	1.00		Ref	1.00		Ref
III/IV	9.99	2.70-36.9	<0.001	5.90	1.89-18.4	0.002
Tumor grade						
1	1.00		Ref	1.00		Ref
2/3/not graded	2.54	0.86-7.71	0.093	1.42	0.48-4.15	0.524
Histology						
Serous	1.00		Ref	1.00		Ref
Non-serous[a]	1.33	0.52-3.40	0.546	1.12	0.44-2.85	0.816
Residual tumor						
0 cm	1.00		Ref	1.00		Ref
<1 cm	3.88	1.50-10.0	0.005	2.06	0.84-5.02	0.112
≥1 cm	2.17	0.92-5.11	0.078	2.24	0.94-5.35	0.069
HE4						
Below cut-off	1.00		Ref	1.00		Ref
Above cut-off	1.31	0.46-3.72	0.612	8.28	3.28-20.9	<0.0001
CA125						
Below cut-off	1.00		Ref	1.00		Ref
Above cut-off	1.29	0.60-2.76	0.513	1.45	0.67-3.17	0.348

[a]Mucinous, endometrioid, clear cell and other. FIGO stage was divided into stage I/II vs. stage III/IV, grade was divided into grade 1 vs. grade 2/3/not graded, and histology into serous vs. non-serous to avoid too many parameters being entered into the Cox model in relation to the number of events. FIGO, International Federation of Gynecology and Obstetrics; HE4, human epididymis protein 4; CA125, carbohydrate antigen 125; HR, hazard ratio; CI, confidence interval; Ref, reference.

≥50% at 3 months (Fig. 4C). However, this group of high-risk patients was small at the 3-month follow-up (n=7). At 6 months, the combination of both markers also predicted PFS P<0.0001 (Fig. 4F), with a median PFS of 56.8 months (95% CI, 28.7-63.2) if both markers were stable, and 11.4 months (95% CI, 10.9-11.9) if both markers increased ≥50%.

On multivariate analysis, CA125 was non-significant at 3- and 6-month follow-up, while HE4 was highly significant at 6-month follow-up, with an HR of 8.23 (95% CI, 3.28-20.9; P<0.0001, Cox regression) (Table II). For the combination of HE4 and CA125 on multivariate analysis, there were too few high-risk patients (n=7) with both markers positive at 3 months for the analysis to be conducted; whereas the HR at 6 months was 7.43 (95% CI, 2.92-18.9; P<0.0001) (data not shown) when both biomarkers increased ≥50%.

Discussion

In June 2008, the HE4 EIA kit (Fujirebio Diagnostics AB) was approved by the FDA to monitor recurrence or progressive disease in patients with epithelial ovarian cancer. In September 2011, the FDA approved marketing of the HE4 test (Fujirebio Diagnostics, Malvern, Pennsylvania) in combination with the CA125 test in the Risk of Ovarian Malignancy Algorithm (ROMA™) as a diagnostic tool for determining the likelihood of malignancy at the time of surgery in women presenting with an ovarian adnexal mass. ROMA™ is a qualitative serum test that combines the results of HE4 EIA, ARCHITECT CA125 II™ (not the same CA125 test used by the present study) and menopausal status into a numerical value and classifies women as being at low- or high-risk for malignant disease. This risk is given as an adjunct to the two test results for CA125 and HE4.

Another possible application of HE4 is during follow-up, which has only been sparsely investigated (26-31). A simple approach is to analyze the marker at the EOT in an effort to identify a group of patients at high risk of early recurrence, an important aspect prompting for further investigation. The results presented herein indicate that analyzing HE4 at this stage is insufficient for reliable classification with regard to the risk of recurrence.

In the current study, measurements of HE4 at the 3- and 6-month follow-ups demonstrated a significant difference in PFS compared with CA125, with considerably higher hazard ratios for HE4. Combination of the two markers, with classification of the patients into a high-risk group if both markers increased ≥50%, did not provide additional value. By reviewing the Kaplan-Meier curves, it appears that HE4

levels are responsible for delineating the large difference in PFS. This was also found to be true on multivariate analysis: CA125 proved not to be significant at 3- or 6-month follow-up, whereas HE4 was highly significant at 6-month follow-up with an HR of 8.23. Similarly, combining HE4 and CA125 in the multivariate analysis, HE4 was revealed to be the important marker, while CA125 appeared not to complement the prognostic value of HE4 during follow-up.

A study by Havrilesky *et al* (26) monitored 27 patients with advanced ovarian cancer subsequent to chemotherapy and evaluated a biomarker panel of which HE4 was one. All 27 patients experienced recurrence following initial response to treatment. The sensitivity for predicting recurrence was 100% for the biomarker panel and 96% for CA125. In 15 patients (56%), ≥1 panel biomarkers were elevated earlier (range, 6-69 weeks) than CA125 and prior to other clinical evidence of recurrence. However, a drawback of this study is the lack of a control group to enable comparison of marker behavior during follow-up in patients with and without recurrence. The current study also demonstrated that, compared to CA125, more patients had elevated HE4 at relapse (or during follow-up), but elevated HE4 was also present in the control group of patients with no clinical detection of relapse.

A study by Plotti *et al* (27) investigated serum CA125 and HE4 levels in 34 patients with radiological suspicion of recurrence and in 34 patients with benign ovarian tumors. The CA125 sensitivity and specificity for detecting recurrent ovarian cancer were 35.29 and 58.82%, respectively. The HE4 sensitivity values were 73.53 and 26.47% when using 70 and 150 pmol/l cut-offs, respectively. HE4 specificity was 100% (all patients in the ovarian cancer group had relapse of ovarian cancer). When combining CA125 and HE4 at a cut-off of 70 pmol/l, the sensitivity in detecting recurrent ovarian cancer was 76.47% with a specificity of 100%. It is difficult to compare these results with the current study, since Plotti *et al* used a patient group with benign tumors for comparison and therefore likely achieved a higher specificity compared to the current results, for which the control group was ovarian cancer patients without recurrence during follow-up.

A relatively recent study by Manganaro *et al* (28) investigated three consecutive serum samples drawn at 3-month intervals from 21 patients with advanced ovarian cancer. In the 9 patients with relapse, an increase in HE4 (>150 pmol/l) was noted in 22, 78 and 89% of the patients according to the time interval from surgery (1-3 months from surgery, 4-6 months and 7-10 months from surgery, respectively). Only 44% of the patients with relapse showed CA125 levels >35 U/ml at 7-10 months from surgery. None of the 12 patients with stable disease had HE4 levels >150 pmol/l, whereas 4 patients had CA125 levels >35 U/ml. These results are in agreement with those of the current study, wherein the predictive value of HE4 also increased with the time interval.

Only a few other studies, which have included ≤20 patients, have investigated HE4 during follow-up, with similar results (29-31).

All of the previous studies are substantially smaller than the current study and do not have a control group of a reasonable number of patients without clinical recurrence/progressive disease. For certain of the studies, it is not clear when follow-up blood samples were taken, and some samples appear to be drawn during the chemotherapy. The material used in the present study was prospectively collected and retrospectively analyzed as part of a prospective marker protocol, and blood tests were recorded regularly during follow-up. The patients were not retrospectively identified, since their recurrence was already known, and a group of patients with no clinical relapse were available for marker comparison. We have previously published the dynamics of HE4 and CA125 during chemotherapy, and therefore this was not in the scope of the present study (32).

The largest of the previously described studies is the study by Plotti *et al* (27). As a criterion for inclusion, these patients had radiological signs of relapse at the time of serum sample collection. Therefore, the sample was drawn at time of diagnosis for recurrent disease and not as part of a follow-up study. The 100% specificity is obvious when all included patients had recurrent disease at sample collection. Furthermore, no comparisons with patients without relapse were performed, and only comparisons with a control group ~30 years younger than an average ovarian cancer cohort, and in which every individual had a benign ovarian tumor.

Early treatment of recurrence may not lead to an improved overall survival time based on therapies available at present (33). Nevertheless, the majority of ovarian cancer patients with advanced disease at diagnosis will relapse after primary treatment, with a dismal prognosis (34). Therefore, investigation of the level of serum markers in patients under monitoring may be essential in distinguishing patients at risk of relapse from those with less aggressive disease. HE4 appears to be a sensitive and specific marker for the detection of recurrence and, in some cases, has the potential to detect recurrence in patients in whom CA125 is negative. However, based on the present results, investigating consecutive blood samples in comparison to a single blood test drawn at the time of diagnosis is not as simple as previously described in the literature. The picture also looks different when marker levels are compared with those of ovarian cancer patients without known relapse, instead of with a group of healthy women. What is clear from the current study and other studies is that there will be cases in which CA125 is not workable and in which HE4 may be a better marker of recurrence.

In conclusion, the results presented here indicate that an early increase of >50% of HE4 in the follow-up period relative to the EOT suggests a high risk of recurrence. This opens the perspective of early treatment. However, the results call for confirmation in a larger number of patient samples.

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