HE4: Clinical applications in gynaecological cancer
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Citation for published version (APA):

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Serum Human Epididymis protein 4 (HE4) as biomarker for the differentiation between epithelial ovarian cancer and ovarian metastases of gastrointestinal origin

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Gynecologic Oncology (136) 2015: 562-6
Abstract

Objective About 5-15% of all malignant ovarian tumours are metastases from other malignancies such as gastrointestinal tumours, breast cancer or melanoma. Also other gynecological tumours can metastasize to the ovaries. It is crucial to differentiate between primary epithelial ovarian cancer (EOC) and ovarian metastases because different treatment is required. The clinical value of Human Epididymis secretory protein 4 (HE4) as a serum biomarker in primary ovarian cancer has been established. The use of HE4 in the differentiation between primary ovarian cancer and ovarian metastases from other malignancies has never been investigated.

Methods HE4, CA125 and CEA were measured in 192 patients with EOC (n=147) or ovarian metastases (n=40). Univariate and multivariate logistic regression analyses were done. Sensitivity, specificity and area under the curve (AUC) were calculated for all markers and ratios hereof using receiver operating characteristics methodology.

Results Median serum HE4 concentration was significantly higher in patients with EOC compared to patients with ovarian metastases (431 pmol/L vs 68 pmol/L, p<0.001). HE4 and CEA were independent factors in differentiating between EOC and ovarian metastases (both p<0.001) while CA125 was not (p=0.33). The HE4².⁵/CEA ratio demonstrated the highest discriminative value (ROC-AUC 0.94) compared to HE4, CEA, CA125 or CA125/CEA ratio (0.88, 0.78, 0.80 and 0.89 respectively) and showed a specificity of 82.5% at set sensitivity of 90% in discriminating EOC from ovarian metastases.

Conclusion HE4 can be used in combination with CEA to make the distinction between EOC and ovarian metastases from gastrointestinal origin.
Introduction

Ovarian cancer is the leading cause of gynaecological cancer-associated death in the Netherlands. (1) About 5-15% of all ovarian malignancies are metastases from another malignancy. The majority of these metastases have a gastrointestinal origin, but also metastases from breast, skin or other gynaecological origin occur. (2-5) Approximately 3-4% of women with colorectal cancer are found to have ovarian involvement. (6) These ovarian metastases can mimic the symptoms of epithelial ovarian cancer (EOC). (7) Moreover, early symptoms may be caused by ovarian metastases instead of the primary tumour, which makes it challenging to differentiate between EOC and ovarian metastases from other malignancies. However, the origin of the tumour is crucial for the subsequent treatment decision-making. (7-8) EOC is treated with either primary or interval cytoreductive surgery and chemotherapy while intra-abdominal metastasized gastrointestinal (GI) tumours may require cytoreductive surgery and HIPEC procedures and other chemotherapy schedules. In case of ovarian metastases from breast cancer, palliative resection may be considered but curative options are often not present.

Currently, CA125 is the most commonly used biomarker in the diagnosis of patients with EOC. Previous research has shown that CA125 alone is not useful in the differentiation between EOC and ovarian metastases, since elevated serum CA125 values were also found in most patients (33-81%) with ovarian metastases and even benign peritoneal diseases. (8-11) The ratio of CA125 and carcinoembryonic antigen (CEA), a glycoprotein that is synthesized in foetal tissues and in colon carcinoma, differentiates better between EOC and ovarian metastases from GI tumours than one of these markers alone. A previous study showed a sensitivity of 73% and specificity of 63% when a cut-off value for the CA125/CEA ratio of 25 was used compared to a sensitivity of 78% and specificity of 50% for CA125 alone. (12)

Serum HE4 is a relatively new biomarker and is useful in the diagnostic work up of patients with a pelvic mass. We performed a previous study that evaluated the role of serum HE4 in the diagnosis of patients with a pelvic mass and found a sensitivity of 90% with specificity of 97% compared to 91% and 65% respectively for serum CA125. (13) No research has yet been done to evaluate whether HE4 can be used to distinguish patients with EOC from those with an ovarian metastasis from another malignancy. In the present study, the use of biomarker HE4 in the preoperative differentiation between EOC and ovarian metastases from gastrointestinal malignancies was evaluated and compared to CA125 and CEA and ratios thereof.
Materials and methods

Patients and tumour characteristics
A retrospective cohort study was performed. We conducted a database search of the Netherlands Cancer Institute (Amsterdam) tumour registration and pathology database to identify women with metastatic ovarian disease that had undergone surgery for the presence of an adnexal mass between January 1994 to January 2012. Women with ovarian metastases originating from cancer of the gastrointestinal tract or breast were included. The cohort of patients with EOC was retrieved from a cohort used in a previous study.(13) Serum samples were collected at time of diagnosis before any surgical procedure. Patients were excluded from the study if they were diagnosed with multiple malignancies at the same time or if they suffered from renal impairment. To this end, serum creatinine level was checked as marker for renal function and a creatinine level above 90 mmol/L was considered as impaired renal function according to our laboratory standards. Patients were considered postmenopausal if they had >1 year of amenorrhea based on personal history. If this information was not available, we used the age of 51 year as a cut-off based on the mean age of menopause in the Dutch population.(14) Treatment of all EOC patients followed the national protocols, but was individualized in metastatic disease after multidisciplinary discussion.

Markers assays
Venous blood samples were collected at time of diagnosis using standard sampling tubes. In the laboratory, the blood was centrifuged and serum was aliquoted in three cryovials. The serum was stored at -30°C until measurement. HE4, CA125 and CEA concentrations in the serum samples of all patients were measured using the electrochemiluminescence immunoassay ‘ECLIA’ on the Cobas®6000 analyser (Roche Diagnostics GmbH, Mannheim, Germany). The upper limit of normal (ULN) for CA-125 was set at <35 kU/L for premenopausal women and <20 kU/L for postmenopausal women. (15)
While serum CA125 values are related to menopausal state, serum HE4 is more related to age.(16) Therefore, cut-off values for HE4 based on age were determined and set at 60 pmol/L for patients younger than 40 years, 75 pmol/L for patients between 40-60 years and finally 90 pmol/L for patients >60 years of age. ULN for CEA was established as 5.0 µg/L for all patients.

Statistical methods
Serum biomarker concentrations are presented in medians values and interquartile ranges (IQR). Mann-Whitney test was used to define significant differences in serum values and age between groups. Number of patients with serum values exceeding...
predefined cut-off values were calculated. Univariate and multivariate logistic regression analysis was performed using the biomarkers (HE4, CA125 and CEA), weighted ratios of those and other factors (age, menopausal state) as predictors to determine independent predictive factors in the differentiation between EOC and ovarian metastases. Serum biomarker concentrations were log transformed before entering the model as they span several orders of magnitude. All test were considered significant at a level of p<0.05.

Next, receiver operating characteristics (ROC) analysis was performed to determine the discriminative potential and determine ideal cut-off values of HE4, CA125, CEA and ratios hereof. Following the example of Moore et al (17) it was decided on forehand that the use of these biomarkers would be considered clinically relevant if the resulting test for discriminating EOC from ovarian metastases would yield a specificity of at least 70% at a set sensitivity of 90%. All analyses were performed using IBM, SPSS (Statistical Package for the Social Sciences) STATISTICS version 22.0.0.0. and R statistical software version 3.0.1.

**Results**

**Patients and tumour characteristics**

In total 192 patients were included; 147 patients had EOC and 45 had ovarian metastases from other malignancies. Most ovarian metastases originated from the gastrointestinal tract (89%, n=40) and the remaining 11% (n=5) were breast cancers. For further analysis we decided to include only cases with ovarian metastases from gastrointestinal origin. One patient with ovarian metastases was excluded because of a serum creatinine value above the predefined threshold.

Patient and tumour characteristics are presented in table 1. The majority of the patients were postmenopausal. Mean age was significantly higher in patients with EOC compared to patients with ovarian metastases (58 versus 52 years, p<0.001). Ovarian metastases of gastrointestinal origin mimicked an EOC in 20% (n=9) of cases and a gynecologist initially operated these patients. Metastases occurred bilateral in 62% of the patients. The histology of all metastatic lesions showed adenocarcinoma of which 12 (30%) were further classified as mucinous. Four tumours contained >10% signet ring cells and were so called ‘Krukenberg’ tumours. The most common histological subtype in the EOC group was papillary serous adenocarcinoma (49%) and the majority was diagnosed at an advanced stage (FIGO III-IV).
### Table 1

Patient and tumour characteristics

<table>
<thead>
<tr>
<th></th>
<th>Ovarian metastases of GI origin (%)</th>
<th>Epithelial ovarian cancer (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. patients</td>
<td>40</td>
<td>147</td>
</tr>
<tr>
<td>Mean age (range)</td>
<td>52 (28-69)</td>
<td>58 (26-86)</td>
</tr>
<tr>
<td>Age groups (years)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;40</td>
<td>5 (12)</td>
<td>9 (6)</td>
</tr>
<tr>
<td>40-60</td>
<td>26 (65)</td>
<td>72 (49)</td>
</tr>
<tr>
<td>&gt;60</td>
<td>9 (22)</td>
<td>66 (45)</td>
</tr>
<tr>
<td>Menopausal state</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Premenopausal</td>
<td>18 (45)</td>
<td>28 (19)</td>
</tr>
<tr>
<td>Postmenopausal</td>
<td>22 (55)</td>
<td>119 (81)</td>
</tr>
<tr>
<td>Lateralism</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unilateral</td>
<td>15 (38)</td>
<td>74 (51)</td>
</tr>
<tr>
<td>Bilateral</td>
<td>25 (62)</td>
<td>32 (22)</td>
</tr>
<tr>
<td>Unknown</td>
<td>40 (27)</td>
<td></td>
</tr>
<tr>
<td>Histology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucinous adenocarcinoma</td>
<td>12 (30)</td>
<td>4 (3)</td>
</tr>
<tr>
<td>Intestinal type adenocarcinoma</td>
<td>19 (48)</td>
<td>0</td>
</tr>
<tr>
<td>Signetring cells</td>
<td>4 (10)</td>
<td>0</td>
</tr>
<tr>
<td>Adenocarcinoma NOS</td>
<td>5 (12)</td>
<td>30 (20)</td>
</tr>
<tr>
<td>Serous papillair adenocarcinoma</td>
<td>72 (49)</td>
<td></td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>7 (5)</td>
<td></td>
</tr>
<tr>
<td>Endometrioïd adenocarcinoma</td>
<td>25 (17)</td>
<td></td>
</tr>
<tr>
<td>Other than EOC</td>
<td>9 (6)</td>
<td></td>
</tr>
</tbody>
</table>

**Abbreviations:** GI; gastrointestinal, NOS; not otherwise specified

### Biomarkers

Table 2 presents the median serum concentrations with IQR and the number of elevated serum values in patients with EOC compared to ovarian metastases compared. The serum concentrations of HE4 in patients with EOC were significantly higher than the serum values in patients with ovarian metastases (median 416 pmol/L versus median 68 pmol/L, p=0.001), while serum concentrations of CEA were significantly higher in patients with ovarian metastases from GI origin (median 6.0 µg/L versus median 1.0 µg/L, p<0.001). Serum HE4 concentrations were elevated in 90% of the patients with EOC and in 43% of the patients with ovarian metastases. An elevated serum concentration of CA125 was found in 90% of patients with EOC and in 65% of the patients with ovarian metastases of GI origin. Serum CEA was only elevated in 10% of patients with ovarian cancer but in 55% of patients with ovarian metastases.
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Table 2

<table>
<thead>
<tr>
<th>Biomarkers in EOC and ovarian metastases of GI tumors</th>
</tr>
</thead>
<tbody>
<tr>
<td>HE4 pmol/L</td>
</tr>
<tr>
<td>n</td>
</tr>
<tr>
<td>----</td>
</tr>
<tr>
<td>Ovarian metastases</td>
</tr>
<tr>
<td>EOC</td>
</tr>
</tbody>
</table>

Abbreviations: GI; gastro-intestinal
*There were four missing values for CEA in the group of subjects with primary ovarian cancer
# Significant difference in serum values between both groups (EOC vs ovarian metastases from GI origin)
IQR: interquartile range; 25th and 75th percentiles

Table 3

Univariate and multivariate logistic regression analysis of all variables followed by bivariate logistic regression analysis of best predictive markers and final univariate model of ratio of HE4 and CEA.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Coefficient</th>
<th>OR</th>
<th>p-value</th>
<th>Coefficient</th>
<th>OR</th>
<th>p-value</th>
<th>Coefficient</th>
<th>OR</th>
<th>p-value</th>
<th>Coefficient</th>
<th>OR</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>0.06</td>
<td>1.06</td>
<td>0.001</td>
<td>0.00</td>
<td>1.00</td>
<td>0.95</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Menopausal state</td>
<td>1.24</td>
<td>3.48</td>
<td>0.001</td>
<td>0.77</td>
<td>2.15</td>
<td>0.41</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log(CA125)</td>
<td>0.72</td>
<td>2.05</td>
<td>&lt; 0.001</td>
<td>0.21</td>
<td>1.23</td>
<td>0.33</td>
<td>X</td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>log(HE4)</td>
<td>1.91</td>
<td>6.76</td>
<td>&lt; 0.001</td>
<td>2.09</td>
<td>8.05</td>
<td>&lt; 0.001</td>
<td>2.51</td>
<td>12.3</td>
<td>&lt; 0.001</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>log(CEA)</td>
<td>-0.83</td>
<td>0.44</td>
<td>&lt; 0.001</td>
<td>-1.00</td>
<td>0.37</td>
<td>&lt; 0.001</td>
<td>-1.00</td>
<td>0.37</td>
<td>&lt; 0.001</td>
<td>X</td>
<td></td>
<td></td>
</tr>
<tr>
<td>HE4/CEA</td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>X</td>
<td></td>
<td></td>
<td>1.00</td>
<td>2.73</td>
<td>&lt; 0.001</td>
</tr>
</tbody>
</table>

Abbreviations: OR; odds ratio
Multivariable logistic regression analyses of all markers (HE4, CA125 and CEA) age and menopausal state revealed that only HE4 and CEA were independent significant factors (both p<0.001) in discriminating between EOC and ovarian metastases from other malignancies (table 3). In the best fitting bivariate logistic regression model using these two markers the coefficients of the log(HE4) and log(CEA) are of opposite sign and of ratio close to 5:2 (table 3). This prompted the construction of an univariate logistic regression model using for each patient the log of the ratio HE4\textsuperscript{2.5}/CEA as the only covariate (table 3). This model, described below, performs as well as the bivariate model and has the advantage of using only one number (the ratio HE4\textsuperscript{2.5}/CEA) per patient. Phrased as a predictive model it results in the following equations:

\[ X = \frac{\text{HE4}^{2.5}}{\text{CEA}} \]

\[ \text{Score} = \exp(-9.6145 + 1.004\ln(X)) \]

\[ P_{\text{PrimOvar}} = \frac{\text{score}}{1 + \text{score}} \]

In this model ‘\( P_{\text{PrimOvar}} \)’ corresponds with the probability that the cancer is of ovarian origin.

At a set sensitivity of 90%, a specificity of 83% (95% CI 67-93%) is reached. Thus, of 143 EOC patients 129 will be correctly classified using this model. This corresponds with a cut-off value for HE4\textsuperscript{2.5}/CEA of 19179 and a value of ‘\( P_{\text{PrimOvar}} \)’ of 0.57.

Specificities at different set sensitivities (85, 90, 95 and 98%) are presented in table 4. ROC analyses were performed for all markers including all patients and showed an AUC for HE4\textsuperscript{2.5}/CEA of 0.94 compared to an AUC of the HE4/CEA ratio of 0.92, and of the CA125/CEA ratio of 0.89 and finally of HE4 alone of 0.88 (figure 1 and 2).

<table>
<thead>
<tr>
<th>Marker</th>
<th>Specificity (%) at set sensitivity of:</th>
</tr>
</thead>
<tbody>
<tr>
<td>HE4\textsuperscript{2.5}/CEA</td>
<td>85%</td>
</tr>
<tr>
<td></td>
<td>90</td>
</tr>
</tbody>
</table>
Serum Human Epididymis protein 4 (HE4) as biomarker for the differentiation between epithelial ovarian cancer and ovarian metastases of gastrointestinal origin

Figure 1
ROC curve with area under the curve of markers HE4, CEA and CA125

Figure 2
ROC curve with area under the curve of ratios of markers HE4, CEA and CA125
Discussion

In this retrospective cohort study we showed that biomarker HE4 can be used, preferably in combination with CEA, to differentiate between EOC and ovarian metastases from gastrointestinal origin. Our data indicate that serum HE4 values are less frequently elevated in patients with ovarian metastases compared to primary ovarian cancer. By using the HE4^{2.5}/CEA ratio instead of HE4 or CEA alone, a larger proportion of patients with EOC will be correctly diagnosed. This ratio showed a better discriminative value than the CA125/CEA ratio evaluated in previous studies. (12,18-19) Sorenson et al (12) evaluated whether CEA in combination with CA125 could differentiate between EOC and ovarian metastases from other malignancies in a group of patients referred for a malignant tumour of unknown origin in the pelvis. They found in their cohort that when CA125/CEA ratio with a cut-off value of 25 is used, 73% of patients with ovarian cancer would be correctly diagnosed preoperatively.

In this study, the formula HE4^{2.5}/CEA showed the best discriminative potential with an AUC of 0.94 and a specificity of 83% at a set sensitivity of 90% for identifying EOC in a group of patients with a malignant ovarian mass using a cut off value of HE4^{2.5}/CEA >19179. A set sensitivity of 90% is relevant from a clinical point of view, because 90% of patients with EOC will be correctly identified. Thus, 129 of 143 patients with EOC and 33 of 40 with ovarian metastases will be correctly classified.

Four of 147 patients with EOC were excluded from this analysis because of missing CEA values. The corresponding cutoff value of HE^{2.5}/CEA of 19179 is derived from developing and testing this ratio in the same patient population. This formula and cutoff value still has to be validated in another patient population.

We presented the formula including HE4 and CEA with the best discriminative potential with respect to the differentiation between EOC and ovarian metastases. From a clinical point of view it can be of benefit to simplify this formula without significantly decreasing the sensitivity and specificity of the model. A simplified version of the formula presented above is HE4^{2}/CEA. Using this simplified version, an AUC of 0.93 is reached with a specificity of 78% at set sensitivity of 90%.

This study is of clinical relevance because a part of the malignant ovarian masses are caused by ovarian metastases from another primary tumour instead of EOC. Moreover, it has been shown that despite careful preoperative examination a presumed malignant ovarian tumour can be mistaken as ovarian metastases of a tumour with another origin. This is not surprising given the considerable overlap in clinical features between EOC and metastases involving the ovaries. (7-8) In both cases inappropriate treatment may be started, that may be ineffective but not without side effects and delay in adequate treatment, with probable deleterious effect on the prognosis of the patient. Although definitive diagnosis is made by
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differentiation between epithelial ovarian cancer and ovarian metastases of gastrointestinal origin

Histopathological examination, it would be helpful if the preoperative differentiation between ovarian metastases and EOC can be improved with the use of biomarkers. This was the first study in which the concentration of the relatively new biomarker HE4 was evaluated in women with metastatic disease in the ovaries from gastrointestinal origin and its role in differentiating this from EOC. Because these metastases are relatively rare, the study group is correspondingly of moderate size. In summary, we developed a formula including HE4 and CEA that can be used to differentiate preoperatively between EOC and ovarian metastases from gastrointestinal origin in case of a malignant pelvic mass. With this formula more patients will be correctly identified. We found that gastro-intestinal tumours comprise the majority of primary tumours with metastatic lesions to the ovaries. To distinguish EOC preoperatively from ovarian metastases, an algorithm using serum biomarkers HE4 and CEA is helpful.

Acknowledgements

The authors would like to thank Fujirebio Diagnostics Inc. for funding this study and Roche Diagnostics for providing the study reagents.

Conflict of interest statement

There are no conflicts of interest for this manuscript.
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1. (NKR) DCR. http://www.cijfersoverkanker.nl consulted on July 25th 2014