HE4: Clinical applications in gynaecological cancer
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CHAPTER 5

HE4 as test for triage of patients with an ovarian mass: a retrospective analysis and introduction to a new prospective trial “HE4 Prediction”

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MR Buist
WJ van Driel
GG Kenter
CAR Lok
Abstract

**Objective** Correct characterisation of an ovarian mass is important for the referral of patients at risk for ovarian cancer to a specialised oncologic centre. Currently, the Risk of Malignancy Index (RMI) with a cut-off value of 200 is used for this purpose. Serum Human Epididymis protein 4 (HE4) is increased in patients with epithelial ovarian cancer (EOC) and to a lesser extent in patients with a benign ovarian mass. Previous studies suggest that serum HE4 has a higher predictive value compared to the RMI. We performed a retrospective study to evaluate the incidence of incorrect referrals and the potential contribution of serum HE4 to improve this suboptimal referral system without compromising the oncologic safety of the patients.

**Methods** HE4 was measured in patients referred to our oncologic centre for treatment of a complex ovarian mass based on RMI between January 2013 until December 2014. Patient and tumour characteristics and the RMI were obtained from the patient charts. Sensitivity and specificity of both methods were calculated. Prediction of RMI and HE4 was also expressed in receiver operating characteristic curves according to the area under the curve (AUC).

**Results** 210 patients were referred for treatment and of 165 patients serum was available for HE4 measurement and further analysis. With a cut-off for RMI of 200, the incidence of needless referrals was 51% (38% benign cysts, 13% borderline cysts). Based on the present data, the addition of HE4 as 2nd step in the triage of patients could decrease the percentage of needless referrals to 30%. The combination of RMI+HE4 has an AUC of 0.82 compared to an AUC of 0.66 of RMI alone to differentiate benign or borderline cysts from an ovarian malignancy.

**Conclusion** The results of this study show that HE4 can decrease the incidence of needless referrals of women with an ovarian mass and this should be investigated in a prospective trial. The protocol of this planned trial is presented.
Introduction

An ovarian mass is a common clinical problem and can affect women of all ages. The a priori chance of a malignant ovarian mass in the general population is low. (1) However, it is important to correctly identify the few malignant masses in order to optimize treatment. Patients with benign cysts are often treated with laparoscopy by their gynaecologist in a general hospital. Patients at risk of a malignant ovarian mass should be referred to a specialised oncologic centre for surgery to allow an immediate staging procedure if necessary. This referral can cause emotional distress and morbidity for patients and an extra (financial) burden for the health care system. To characterise an ovarian mass preoperatively as benign, borderline or malignant, various biomarkers and prediction models have been studied. Currently, the RMI score is often used to estimate the risk of malignancy. The RMI is developed by Jacobs et al. in 1990 and gives an estimation of the risk of malignancy based on serum Cancer Antigen 125 level (CA125), specific ultrasound factors and menopausal state.(2) The most commonly used cut-off value for RMI is 200. Based on a recent review and meta-analysis, the RMI has a pooled sensitivity of 72% and specificity of 92% for differentiating between a benign and malignant ovarian mass.(3) In the Netherlands, patients with a complex ovarian mass and a RMI $\geq$200 are referred to a specialised oncologic centre for further treatment. Although the RMI includes ultrasound features, serum CA125 value has significant influence on the final score. Serum CA125 is frequently found to be normal in patients with early stage EOC. Also, it can show false-positive results in benign masses leading to an increased RMI and consequently needless referrals.(4) Therefore, there is need for a new biomarker that can complement CA125 or the RMI in the diagnosis of patients with an ovarian mass. (5)

Evaluation of gene expression profiles in EOC showed that the WFDC2 gene encoding a glycoprotein identified as HE4 is amplified in EOC. Since then, more studies have shown that HE4 has an increased specificity compared to CA125 and a comparable sensitivity for the diagnosis of EOC.(6,7) HE4 can be combined with CA125 in a prediction model called ROMA. The sensitivity of this score is calculated to be 94% at a predefined specificity of 75% by Moore et al.(8) Ultrasound features are not used in this algorithm. In contrast, models such as the IOTA-rules and Simple Rules only use ultrasound features in their calculation and do not use serum biomarkers.(3) We performed a retrospective study to evaluate the incidence of needless referrals and the potential contribution of serum HE4 to improve the current referral system.
Materials and methods

Patient selection
Patients that were referred with a complex ovarian mass to the Centre for Gynaecologic Oncology Amsterdam (CGOA) from 2013 to 2014 were included. All preoperatively obtained serum samples were collected for the measurement of serum CA125 and HE4 concentration. Patient and tumour characteristics were collected by retrospective chart review. Patients were excluded if there was no definitive histological diagnosis available or in case of an impaired renal function at time of serum collection, because this could have influenced the serum HE4 concentration.(9,10) Serum creatinine level >100 mmol/L was used as a cut-off value to define an impaired renal function.

Risk of Malignancy Index
The RMI was obtained from the patient charts of the referring hospitals. In case this was not available, the RMI was calculated from the necessary parameters (menopausal state, serum CA125 and ultrasound features) obtained from the referring hospital charts. The RMI was defined as described by Tingulstadt et al and a cut-off value of 200 was used to identify patients at risk of EOC.(2)

Serum CA125 and HE4 measurements
Venous blood samples were collected preoperatively using standard sampling tubes without additives. Blood samples were centrifuged and serum was stored at −80°C until measurement. Serum HE4 concentration was measured in the laboratory of the Netherlands Cancer Institute/Antoni van Leeuwenhoek (NKI-AVL). Serum HE4 levels were analysed using the electrochemiluminescence immunoassay ‘ECLIA’ on the Cobas®6000 analyser (Roche Diagnostics GmbH, Mannheim, Germany). Cut-off values for serum HE4 were based on age and set at 60 pmol/L for patients younger than 40 years, 75 pmol/L for patients between 40 and 60 years and finally 90 pmol/L for patients >60 years of age. (11)

Statistical analysis
Sensitivity and specificity results were calculated for both RMI and serum HE4. ROC curves with corresponding Area Under the Curve (AUC) were reconstructed to compare the diagnostic accuracy of RMI and HE4. All analysis were performed using IBM, SPSS (Statistical Package for the Social Sciences) Statistics version 22.0.0.
Results

A total of 210 patients were included in analysis. Serum of 165 patients was available for the measurement of HE4 concentration. Of all patients, 178 (85%) had a RMI ≥200. The remaining 32 patients (15%) were referred despite an RMI <200 because of suspicious ultrasound findings or extensive co-morbidity that required specialised perioperative care. An overview of patient and tumour characteristics is given in table 1. Two groups are made based on the RMI. In total, a benign mass was diagnosed in 85 patients (41%), a borderline mass in 32 patients (15%) and a malignancy in 93 patients (44%).

Of all patients that were referred because of an RMI ≥200, final histological diagnosis showed an ovarian malignancy in 87 patients (49%), of which the majority were a serous type adenocarcinoma. A benign or borderline ovarian mass was diagnosed in 67 (38%) and 24 (13%) patients, respectively.

Table 1

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>RMI ≥200 n=178 (%)</th>
<th>RMI &lt;200 n=32 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean age (SD)</strong></td>
<td>60.8 (11.3)</td>
<td>57 (11.2)</td>
</tr>
<tr>
<td><strong>Postmenopausal</strong></td>
<td>148 (83%)</td>
<td>23 (72%)</td>
</tr>
<tr>
<td><strong>Histological diagnosis</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Benign</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serous cystadenoma</td>
<td>22 (32%)</td>
<td>6 (33%)</td>
</tr>
<tr>
<td>Fibroma</td>
<td>15 (22%)</td>
<td>2 (11%)</td>
</tr>
<tr>
<td>Mucinous cystadenoma</td>
<td>12 (18%)</td>
<td>4 (22%)</td>
</tr>
<tr>
<td>Endometriosis</td>
<td>6 (9%)</td>
<td>0</td>
</tr>
<tr>
<td>Leiomyoma</td>
<td>3 (5%)</td>
<td>3 (17%)</td>
</tr>
<tr>
<td>Brenner tumor</td>
<td>3 (5%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Other</td>
<td>3 (5%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td>Infection/inflammation</td>
<td>2 (3%)</td>
<td>0</td>
</tr>
<tr>
<td>Teratoma</td>
<td>1 (2%)</td>
<td>1 (6%)</td>
</tr>
<tr>
<td><strong>Borderline</strong></td>
<td>24 (13%)</td>
<td>8 (25%)</td>
</tr>
<tr>
<td>Serous borderline tumor</td>
<td>13 (54%)</td>
<td>2 (25%)</td>
</tr>
<tr>
<td>Mucinous borderline tumor</td>
<td>11 (46%)</td>
<td>6 (75%)</td>
</tr>
<tr>
<td><strong>Malignant</strong></td>
<td>87 (49%)</td>
<td>6 (19%)</td>
</tr>
<tr>
<td>Serous papillary adenocarcinoma</td>
<td>41 (47%)</td>
<td>0</td>
</tr>
<tr>
<td>Mucinous adenocarcinoma</td>
<td>12 (14%)</td>
<td>0</td>
</tr>
<tr>
<td>Endometrioid adenocarcinoma</td>
<td>12 (14%)</td>
<td>0</td>
</tr>
<tr>
<td>Clear cell carcinoma</td>
<td>6 (7%)</td>
<td>0</td>
</tr>
<tr>
<td>Adenocarcinoma NOS</td>
<td>3 (3%)</td>
<td>1 (17%)</td>
</tr>
<tr>
<td>Ovarian metastasis</td>
<td>3 (3%)</td>
<td>1 (17%)</td>
</tr>
<tr>
<td>Other</td>
<td>10 (12%)</td>
<td>4 (67%)</td>
</tr>
</tbody>
</table>

Abbreviations: RMI; risk of malignancy index, NOS; not otherwise specified
In the group of patients that were referred despite an RMI<200, the majority was diagnosed with a benign ovarian mass (n=18, 56%). Six patients (19%) in this group were diagnosed with an ovarian malignancy. Final histological diagnosis in these patients revealed 3 granulosacel tumours, 1 carcinosarcoma, 1 undifferentiated carcinoma and 1 metastatic tumour of gastrointestinal origin. In four patients (4%) in total the ovarian mass was not of ovarian origin but a metastasis of a primary gastrointestinal tumour.

**HE4**

HE4 was elevated in 42% of the total group with available HE4 data. An elevated serum HE4 concentration was found in only 19% of patients with benign ovarian mass (table 2). In contrast, serum CA125 was elevated in 72% of patients with a benign ovarian mass. Table 3 illustrates HE4 results in subgroups based on the RMI. Of all patients with an RMI≥200 and available HE4 data, 69 patients (48%) were diagnosed with a benign or borderline mass and thus misclassified. In subgroup of patients with RMI≥200, elevated HE4 concentrations were measured in 21 patients (30%) with a benign or borderline mass. In the majority of these patients (n=48, 70%) a normal HE4 concentration was found (table 3b). In 82% of patients with an ovarian malignancy both RMI and HE4 were elevated. Normal HE4 concentrations was measured in 13 patients (18%) with an ovarian malignancy. In literature, borderline tumours are sometimes considered (pre)malignant. It is a matter of debate whether these patients should be treated in an oncologic center. Even if we classify borderline tumors as malignant tumors, still 36% of the patients were misclassified with RMI and diagnosed with a benign ovarian mass. Serum HE4 as second step decreased this percentage to 19% (results not shown).

### Table 2

Overview of HE4 results divided by definitive histological diagnosis

<table>
<thead>
<tr>
<th>Test</th>
<th>Benign n=63 (%)</th>
<th>Borderline n=23 (%)</th>
<th>Malignant n=79(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HE4</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal(^2)</td>
<td>51 (81%)</td>
<td>10 (44%)</td>
<td>16 (20%)</td>
</tr>
<tr>
<td>Elevated</td>
<td>12 (19%)</td>
<td>13 (56%)</td>
<td>63 (80%)</td>
</tr>
</tbody>
</table>

\(^2\) Age-defined cut-off concentrations were used to identify ‘normal’ and ‘elevated’ values.
HE4 as test for triage of patients with an ovarian mass: a retrospective analysis and introduction to a new prospective trial "HE4 Prediction"

Table 3
Serum HE4 results in patients with RMI<200 (3a) and RMI≥200 (3b) with available HE4 results (n=165). Percentages represent sensitivity and specificity of HE4.

3a

<table>
<thead>
<tr>
<th>RMI&lt;200</th>
<th>Benign/Borderline n=17 (%)</th>
<th>Malignant n=5 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal HE4</td>
<td>13 (76%)</td>
<td>3 (60%)</td>
</tr>
<tr>
<td>Elevated HE4</td>
<td>4 (24%)</td>
<td>2 (40%)</td>
</tr>
</tbody>
</table>

3b

<table>
<thead>
<tr>
<th>RMI ≥200</th>
<th>Benign/Borderline n=69 (%)</th>
<th>Malignant n=74 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal HE4</td>
<td>48 (70%)</td>
<td>13 (18%)</td>
</tr>
<tr>
<td>Elevated HE4</td>
<td>21 (30%)</td>
<td>61 (82%)</td>
</tr>
</tbody>
</table>

ROC analyses showed that the potential to discriminate between a benign or borderline mass versus an ovarian malignancy, is higher when RMI and HE4 are combined (AUC 0.82, 95% CI 0.75-0.89) compared to RMI alone (AUC 0.66, 95% CI 0.57-0.75) (figure 1).

Figure 1
ROC curve of RMI alone compared to RMI+HE4 illustrating the value to discriminate between benign/borderline versus malignant ovarian mass in patients with RMI≥200

ROC curve: RMI alone
- Sensitivity
- Specificity

ROC curve: RMI+HE4
- Sensitivity
- Specificity

AUC values:
- RMI: 0.66 (0.57-0.75)
- RMI+HE4: 0.82 (0.75-0.89)
Conclusion

In this retrospective study, we analysed the diagnostic accuracy of serum HE4 in a selected group of patients who were referred from a secondary care setting to a specialised oncologic centre because of a suspected EOC based on the RMI or other clinical features. Final histological diagnosis confirmed a malignancy in 49% of patients with an RMI $\geq 200$. Of the total group, including patients with missing HE4 data, in 51% final pathological diagnosis revealed a benign or borderline ovarian mass and these patients were thus incorrectly referred. These results illustrate the relatively low specificity of the RMI score. In contrast, in patients with an RMI $\geq 200$ and a benign or borderline ovarian mass, serum HE4 was normal in 70% of patients. The combination of RMI and HE4 improves the diagnostic accuracy compared to RMI alone. Probably a two-step triage system where both tests are combined will lead to a more accurate preoperative risk selection of patients that present with a complex ovarian mass in a secondary care setting. Based on our results, HE4 as second step could potentially decrease the percentage of incorrect referrals from 48% to 30% of patients.

The type of clinical setting should be included in the decision for a certain test, because each setting (primary, secondary, tertiary) demands different test characteristics depending on the population that is tested. Also, in general most tests do not perform equally in each setting because the positive predictive value depends on the prevalence of disease in a specific population. In a secondary setting, not only a test with a high sensitivity but also with a high specificity is needed to warrant an optimal referral system for patients that need to be referred to a tertiary care setting. The results of this retrospective study illustrate the lack of specificity of RMI and support the use of other markers or prediction models, for example HE4. However, these numbers, need to be interpreted with caution since the study group includes a selected group of patients and the results cannot be extrapolated to all women with a complex ovarian mass. It is unknown how many women with an RMI $< 200$ (and unknown HE4 concentration) underwent surgery in local hospitals. So, only a part of the total population has been investigated. Therefore, these results cannot yet be used to change the current referral policy. Also, it is unknown whether other cut-off values for both tests could further increase the diagnostic accuracy, certainly in case of contradictory results of RMI and HE4. The results of this study in addition to previous literature forms the basis for a prospective trial.
HE4 Prediction: a prospective evaluation of Human Epididymis protein 4 (HE4) as predictor of malignancy in patients with a complex ovarian mass.

Objective

The aim of this study is to assess the additive value of serum biomarker HE4, as second step after the RMI score, in the triage of patients with a complex ovarian mass. Secondary outcomes are cost-effectiveness and quality of life.

Methods and design

This study is a multicentre prospective observational cohort study performed in the Centre of Gynaecologic Oncology Amsterdam (CGOA) and affiliated hospitals. All patients with a complex ovarian mass, evaluated with RMI, will be included. According to current national protocol, patients with an RMI <200 will undergo surgery in one of the affiliated hospitals, while patients with an RMI score ≥200 will be referred to an oncologic centre. Serum HE4 will be determined in a preoperative obtained serum sample. A flow diagram of the study protocol is shown in figure 2.

Eligibility criteria

Patients are eligible for inclusion if they are ≥18 years of age, have understanding of the Dutch or English language and have a complex ovarian mass. Also, according to the WHO performance status, they should be fit for a surgical intervention to obtain a histological sample for final diagnosis. Patients with a decreased Glomerular Filtration Rate (GFR) (<60ml/min/1,73m2), with other malignancies or with obvious advanced disease are excluded.

Patient recruitment, data and sample collection

All patients that visit one of the affiliated hospitals of the CGOA with a complex ovarian mass and who fit the inclusion criteria, will be asked for informed consent. At time of serum collection for measurement of CA125, three extra tubes will be taken. These samples will be send to the NKI-AVL for the measurement of serum HE4 value according to the process described in our retrospective analysis. The results will not influence the decision for referral to an oncologic centre or not. Depending on the experience of the ultrasound examiner, the Simple Rules criteria will be collected. Other relevant clinical data that will be collected are: age, menopausal state, history of ovarian cancer, smoking status, body mass index and findings at
gynaecological examination. Collection of these variables enable calculation of the IOTA score (LR1 and LR2). Final pathological diagnosis is collected from the hospital were the patient is operated. In case of doubt, a dedicated gynaecologic pathologist will perform review of the slides.

**Secondary outcome, cost-effectiveness of HE4 measurement**

The key question in the economic evaluation is to assess whether measurement of HE4 can reduce the referral of patients with benign disease, decreasing health care costs, despite extra costs of the HE4 measurement. We hypothesize, however, that additional costs of HE4 measurement are greatly offset by the reduced number of futile referrals. We will hypothesize the cost of HE4 measurement at each referral centre. We will compare the costs for patient care, at current protocol based on the RMI, and at our new strategy combining RMI and HE4 measurement. Duration of hospital stay and costs of the final treatment will be taken in consideration.

**Secondary outcome, quality of life**

Referral to a specialised oncologic centre might influence the perception of anxiety and the level of stress. Standardised questionnaires will be used to measure anxiety, stress and patients satisfaction upon entry of the study and six weeks after surgery.
Patients with a complex ovarian mass

Informed consent, n>66

TVE; RMI criteria and Simple Rules*
Clinical data

Serum CA125
Extra sample for serum HE4 measurement

RMI < 200*

Surgery in affiliated hospital

RMI ≥ 200*

Referral to oncologic centre

Primary outcome: additive value of serum HE4 as 2nd step in the triage of patients with an ovarian mass
Secondary outcome: cost-effectiveness, quality of life

# If possible
* In case of other factors suspected of malignancy a patient can be referred to a tertiary center according to the judgement of the gynaecologist despite a RMI score <200.

Simple rules

B-criteria: unilocular, presence of a solid component <7mm, acoustic shadows, smooth multilocular tumor <100mm diameter, no blood flow

M-criteria: irregular solid tumor, ascites, ≥4 papillary structures, irregular multilocular solid tumor ≥ 100mm, very strong blood flow (color score 4)
Statistical analysis

Power calculation is based on results from previous literature and our retrospective analysis. The incidence of ovarian cancer in all women with an ovarian cyst is estimated to be 5%. From previous literature we know that the sensitivity and specificity of the RMI score are 72%-79% and 92%, respectively.(3,6) We aim to find an increase of sensitivity with the addition of HE4 to the RMI of 10% without handing in on specificity.

Thus, our goal is to reach a sensitivity of ≥85% for both tests combined. If we assume that serum HE4 and the RMI of each patient is statistically independent, we can compute a sensitivity of 94% if either the HE4 or RMI is increased. However, this means that the specificity decreases considerably. A combination of RMI and HE4 where for example a variable thresholds for HE4 is used based on the value of the RMI, could increase the sensitivity of the test while maintaining a high specificity. Elaborating on the maximum achievable sensitivity of 94% as mentioned before, we believe that a sensitivity of 85% for a test that combines RMI and HE4 is realistic and clinical relevant.

Assuming a true sensitivity of 85% for a suitable test combining RMI and HE4, we need to include 66 patients with EOC to reach a power of 80% to find that the confidence interval around the observed sensitivity of the new combination lies entirely above 72%, which is the sensitivity found in the literature for the current test based on RMI alone.

At this moment, it is unknown how many patients are presented in a general hospital with a complex mass and an RMI <200. With an incidence of malignancy of 40% in all referred patients, continuing inclusion of patients until the number of 66 patients with malignant disease is reached, which will provide us enough patients with benign masses to give reliable estimates of secondary outcome measures such as specificity, positive and negative predictive values of the combination of RMI and HE4.
Economic evaluation

The aim of the economic evaluation is to assess whether use of HE4 as 2nd step after the RMI score can reduce the number of patients that need to be referred to a tertiary centre and associated costs. A strategy that reduces the amount of unnecessary referrals without resulting in more patients that need a second surgical procedure because they are incorrectly not referred, is preferable. Costs that will be taken into consideration are costs for measurement of serum HE4, referral to a tertiary centre and accompanying extra diagnostic tests and appointments, costs for (second) surgery, operation time and hospital admission days.

Discussion

Evaluation of a complex ovarian mass and the decision for referral to a specialised oncologic centre with the RMI, is still considered standard protocol in many hospitals. However, using RMI alone results in unnecessary referral of 51% of patients with a benign or borderline disease. A triage method that leads to a minimal percentage of unnecessary referrals but on the other hand identifies those patients at risk of EOC who benefit from a referral to a specialised oncologic centre, needs to be pursued. Research over the last couple of years has shown that more patients with a complex ovarian mass are accurately diagnosed preoperatively with serum HE4. (6,7) To completely replace the RMI by HE4 would exclude ultrasound findings from the diagnostic work-up, while this is a standard step in the assessment of a complex ovarian mass in the Netherlands and provides the gynaecologist valuable information. Besides, the RMI has a high sensitivity that is comparable to that of serum HE4. An increase in specificity is however preferred. To achieve this, a combination of RMI and HE4 as preoperative test seems a feasible option for daily clinical practice. This should be investigated in a prospective trial in a general population and the results have to be compared to other ultrasound based prediction models such as IOTA and simples rules.

Acknowledgements

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