HE4: Clinical applications in gynaecological cancer
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CHAPTER 1

General introduction and outline of the thesis
CHAPTER 1

GENERAL INTRODUCTION

Cancer of the gynaecological tract can affect all women. Figure 1 illustrates the incidence of different types of gynaecological cancer. Of all types, the most common is endometrial cancer (EC) originating in the uterine cavity. Second most common, but causing the most gynaecological cancer-related deaths is epithelial ovarian cancer (EOC). Together, EC and EOC, comprise the two most important types of gynaecological cancer.

Figure 1
Incidence of all types of gynaecological cancer over the last 15 years (Netherlands Cancer Registration)

Figure 2 illustrates the growing number of publications concerning EOC (fig 2a) and EC (fig 2b). Most research is focused on the etiology, diagnosis and treatment of EOC. Despite this extensive efforts, the incidence and mortality rate of ovarian cancer has remained stable in the Netherlands (figure 3) and worldwide. In general, there are three points of engagement for research that could result in a decrease in morbidity and mortality: 1) early detection of ovarian cancer in an asymptomatic general population or a population at risk for ovarian cancer, 2) adequate preoperative diagnosis of ovarian cancer in the presence of an ovarian mass and 3) optimal surgical and chemothereapeutical treatment in advanced stage ovarian cancer by gynaecologic oncologists and medical oncologists.
Figure 2a
Overview of publications on ‘ovarian cancer’ from 1990-2015. (pubmed.gov)

Figure 2b
Overview of publications on ‘endometrial cancer’ from 1990-2015 (pubmed.gov)
In contrast to ovarian cancer, endometrial cancer is often detected early and prognosis is favourable in these patients. One of the important questions to be answered in order to reduce morbidity in endometrial cancer patients, is how to select patients that may benefit from lymph node dissection or adjuvant therapy.

EPITHELIAL OVARIAN CANCER

Incidence and survival
EOC is diagnosed in ±1200 patients each year in the Netherlands. In 2014, a total of 1025 women died of the consequences of EOC. Due to the late onset and non-specific nature of symptoms, the majority of women (70%) is diagnosed with advanced stage disease (FIGO stage III-IV). Standard treatment of advanced EOC consists of cytoreductive surgery in combination with (neo)adjuvant chemotherapy which leads to a five-year survival of only 20-45%. Gene profiling studies have identified specific mutations that harbour an increased life-time risk of developing EOC. A hereditary predisposition is found in 5-10% of patients diagnosed with EOC and is mainly caused by a mutation in the BRCA1 or BRCA2 gene. So far, screening strategies in women at risk for EOC have not proven to be effective yet in terms of a reduction of the mortality rate.
Current diagnosis and management

In general, the a priori chance of a malignant origin of an ovarian mass is low, but the correct identification of patients at risk for EOC is important for subsequent management.(4) The diagnostic evaluation of an ovarian mass consists of a combination of physical and gynaecological examination, measurement of serum tumour marker cancer antigen 125 (CA125) and (transvaginal) ultrasound. In case of clinically early stage EOC, surgical staging is performed according to the International Federation of Gynaecology and Obstetrics (FIGO) rules for classification. (2) In advanced disease, computed tomography (CT-scan) and a cytological or histological sample complete the diagnostic work-up.

Serum tumour marker CA125 is an established serum biomarker in the diagnosis of EOC. Serum CA125 is only elevated in 50% of patients with low stage EOC resulting in a limited sensitivity. The effectiveness of CA125 in the diagnosis of EOC is compromised by the fact that CA125 is elevated in many benign gynaecological diseases such as endometriosis, uterine fibroids, cystadenomas and pelvic inflammatory disease, resulting in a reduced specificity of 75%.(5) Serum CA125 is incorporated in the Risk of Malignancy Index (RMI): a simple scoring system based on menopausal state, ultrasound and CA125 to evaluate the risk of EOC in women with a complex ovarian mass.(6,7) The RMI is defined as $U \times M \times CA125$, where $U$ = ultrasound score, $M$ = menopausal status and $CA125$ = serum level in kU/L (figure 4). In the Netherlands, a cut-of value of 200 is used to define patients with an increased risk of EOC who need to be referred to a specialised oncologic centre for further treatment. Characterisation of a benign mass is important to avoid unnecessary referral, second surgical procedure with coinciding morbidity and a burden for the patient and health care system.

Although the RMI is comprised of a combination of factors, serum CA125 has a major influence on the final score. This limits the test performance of the RMI and it is therefore suboptimal for the early detection of women with ovarian cancer. Other prediction rules that have been developed for the diagnosis of patients with an ovarian mass are the Simple Rules and IOTA score. Unlike the RMI, the Simple Rules do not include a serum biomarker but are based on five ultrasound features to distinguish a malignant ovarian mass and five ultrasound features that predict a benign tumour. (8,9) The International Ovarian Tumour Analysis group also developed two logistic regression models (LR1 and LR2) for the preoperative assessment of an ovarian mass. Twelve and respectively six independent prognostic demographic and ultrasound features were selected and comprise together the LR1 and LR2. (10) Comparison of the RMI, Simple Rules, LR1 and LR2 for the preoperative characterisation of an ovarian mass showed an advantage of the Simple Rules and LR2 over the RMI. (11) Nevertheless, these models have not been implemented into the Dutch national guidelines yet.
Figure 4
Risk of Malignancy Index score (Tingulstadt et al 1999)(7)

<table>
<thead>
<tr>
<th>Ultrasound criteria (U)</th>
<th>Menopausal state (M)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multilocular</td>
<td>Premenopausal 1</td>
</tr>
<tr>
<td>Solid areas</td>
<td>Postmenopausal 3</td>
</tr>
<tr>
<td>Bilaterality</td>
<td></td>
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<tr>
<td>Ascites</td>
<td>Serum CA125 (kU/L) - - - -</td>
</tr>
<tr>
<td>Intra-abdominal metastases</td>
<td></td>
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<tr>
<td>Total</td>
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<td>Score</td>
<td>0 1</td>
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<td>≥ 2 3</td>
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</tbody>
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\[ \text{RMI} = U \times M \times \text{CA125} \]

OVARIAN METASTASES

Besides the correct differentiation between a benign and malignant mass of ovarian origin, it is equally important to exclude an extra-ovarian origin of the tumour. The ovaries are a preferred localisation for metastases of some malignant tumours and in 10-15% of patients an ovarian malignancy represents a metastatic lesion of another primary tumour instead of primary ovarian cancer. The majority of these ovarian metastases have their origin in the gastrointestinal tract or breast and they can mimic the symptoms of a primary ovarian malignancy. (12,13) Sometimes the metastatic tumour is detected before the primary tumour, because the metastasis causes complaints earlier than the primary tumour. The differentiation is important for adequate treatment decisions, but current clinical and radiological tests are not always conclusive. Biopsies can be helpful, but definitive diagnosis is often based on pathological review of surgical tissue specimen. Besides morphologic features, immunohistochemistry (IHC) markers can be used in addition to differentiate between tumour types. Frequently used markers for this purpose are CA125, carcino-embryonic antigen (CEA), Wilms tumour gene-1 (WT1), oestrogen receptor (ER), cytokeratin (CK) 7 and 20 and PAX8. (14) Despite these IHC markers it can still be challenging to define the origin of an ovarian tumour.
ENDOMETRIAL CANCER

Endometrial cancer (EC) is the most common type of gynaecological cancer in the Netherlands (figure 1). In contrast to EOC, the majority of patients are diagnosed in an early stage (FIGO stage I) and have a generally good prognosis (five-year survival 91%). Five-year survival rates are much lower when the disease extents into the cervix (FIGO stage II), beyond the uterus into the ovaries or regional lymph nodes (FIGO stage III) or if there are distant metastases (five year survival rates of 78%, 57% and 23% respectively).(15) EC is surgically staged according to the 2009 FIGO staging system. (16) EC is historically classified into one of two following clinicopathological categories: type I include the most common low-grade endometrioid adenocarcinoma (80-90%) and type II comprises high-grade endometrioid adenocarcinoma and non-endometrioid subtypes such as serous, clear cell, carcinosarcomas and undifferentiated carcinomas (10-20%).

Predictive and prognostic factors
Type I EC has a better prognosis compared to type II. However, sometimes a patient with a type I classified tumour harbours a worse outcome than expected. This is probably due to the presence of other histopathological risk factors that are not included in the subdivision of type I and type II EC. Important prognostic factors in EC are age, histological subtype, tumour grade, the extent of myometrial invasion, lymph vascular space invasion (LVSI) and lymph node metastases. Lymph node status is a major prognostic factor of survival in EC and is related to histological type, myometrial invasion and tumour grade. (17,18)
One of the main challenges for clinicians, is to identify patients preoperatively that might benefit from systematic lymphadenectomy. (19–21) Information on tumour grade and myometrial invasion is obtained by endometrial biopsy and radiological imaging. Discrepancies between preoperative assessment and final pathology occur regularly. (22–24) A serum biomarker could potentially improve the preoperative assessment of the extent of EC and to guide treatment planning. Serum CA125 is not effective for this purpose since it is only elevated in a minority of patients with EC. (25)
**HUMAN EPIDIDYMIS PROTEIN 4**

Simultaneously with the development of new prediction rules and models, search for new serum biomarkers to complement or replace CA125, has rapidly gained interest. Among all biomarkers evaluated, Human Epididymis protein 4 (HE4) is one of the most promising.(26) The gene encoding for the HE4 protein, *WFDC2*, is located on the chromosome 20q13. HE4 is a member of the whey-acid four-disulphide core (*WFDC*) protein family including two protease inhibitors elafin and secretory leukocyte protease inhibitor (SLPI). The function of the HE4 protein remains largely unknown and also the secretion in other body fluids is not completely elucidated yet.(27) HE4 was initially identified exclusively in the distal part of the epididymis and vas deferens. Gene expression profiles showed overexpression of the *WFDC2* gene in ovarian carcinoma cells. (28) This finding was confirmed in studies showing that HE4 protein expression in healthy tissue was restricted to the reproductive tract and respiratory epithelium and overexpressed in EOC. (29–31) In addition, HE4 gene expression, tissue immunoreactivity and secretion in the bloodstream was highest in EOC compared to for example gastrointestinal malignancies or breast cancer. (31–33) Serum HE4 concentration is less frequently elevated in benign gynaecological conditions compared to serum CA125, with a consequently higher specificity; a promising result that suggests a role for HE4 in the diagnosis of ovarian and endometrial cancer. (34–37)

**AIMS AND OUTLINE OF THE THESIS**

**Aims**

In this thesis we aim to define the role of HE4 in different clinical settings to determine its place in current diagnosis and treatment protocols in EOC and EC. We focus on the early detection and preoperative diagnosis of EOC and differentiation of primary EOC from ovarian metastases and how to select patients with EC that benefit from lymph node dissection.

**Outline of the thesis**

Research focused on new biomarkers and prediction models is only useful if there is a need for it in daily clinical practice. Therefore, we conducted a nationwide survey among gynaecologists and gynaecologists in training about the current diagnostic work-up in patients with an ovarian mass and evaluated the perceived need for new biomarkers and prediction models. The results are described in chapter 2.
In **chapter 3** we investigated serum biomarker HE4 in our own patient population and attempted to develop a prediction model including HE4 and radiologic features. The data used in this study are also used in an international multicenter study that is described in **chapter 4**. Elaborating on the results described in chapter 3, we evaluated the use of serum biomarker HE4 as second step after calculating the RMI, for the preoperative differentiation of an ovarian mass. The results of this study are reported in **chapter 5** and a proposal for a prospective study is described. Even better than the early diagnosis of patients with an ovarian mass, is detecting ovarian abnormalities before the onset of symptoms or before it can be seen on ultrasound in a population at risk for EOC. For instance, in patients with a germline BRCA1 or BRCA2 mutation. So far, there is a lack of effective screening strategies for women at risk for ovarian cancer. Therefore, we analysed serial HE4 measurements in patients at risk of developing EOC. The results hereof are described in **chapter 6**.

We know from other screening programs that screening test do not necessarily need to be restricted to serum samples, but can also be performed in other body fluids such as cervical swabs. Therefore, we analysed the secretion of the HE4 protein in cervical mucus and other body fluids in a group of healthy women and patients with a benign, borderline or malignant ovarian mass. The results on this topic are reported in **chapter 7**.

A small number of ovarian malignancies do not primary develop in the epithelium of the ovary, but develop in other organs such as the gastrointestinal tract and metastasize to the ovary. In **chapter 8** we focus on the preoperative differentiation between primary ovarian cancer and ovarian metastases of gastrointestinal origin and evaluate the role of serum HE4 in this diagnostic dilemma. Many patients with advanced EOC or metastatic disease in the ovaries present with ascites. Therefore, we also investigated if HE4 can be used as immunocytochemistry marker in ascites and if this contributes to the differentiation between malignant ascites from EOC or gastrointestinal origin. These results are reported in **chapter 9**.

In EOC serum biomarkers are commonly used. In other gynaecological malignancies, e.g. EC, the role of serum biomarkers is less well established. Because most patients with EC develop symptoms in an early stage, the role of a new serum biomarker is more focused on risk stratification of patients to guide surgical planning. We evaluated serum HE4 as prognostic marker in endometrial cancer in **chapter 10**.

The content of this thesis and recommendations for further research are discussed in **chapter 11**.
References


