HE4: Clinical applications in gynaecological cancer
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CHAPTER 11

General discussion and future perspectives
National Institute of Health defined a biomarker or ‘biological marker’ as ‘a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacological responses to a therapeutic intervention’. (1) Biomarkers are currently widely used in research and daily clinical practice. Most biomarkers are detected in serum, which makes them not only objective and reproducible but also easy applicable and minimally invasive. With respect to malignant disease, including ovarian cancer, a serum biomarker can be valuable for screening, diagnosis, predicting treatment response and monitoring treatment, follow-up and predicting prognosis. Each objective requires different characteristics of a biomarker and there is usually a trade-off between sensitivity and specificity measures.

For oncological screening purposes in an asymptomatic population, a biomarker with high sensitivity and few false-negative test results is needed. Thereby a relatively low specificity is accepted and thus some patients will undergo unnecessary additional testing. Theoretically this could lead to stress and anxiety in these patients. However, it has been shown in the UKCTOCS trial that the anxiety level of women who have been subjected to ovarian cancer screening is not increased, so the possible psychological burden should not be a holdback. (2)

In a primary care setting, where the majority of woman with an ovarian mass do not have cancer (3), a biomarker that is used for diagnosis requires especially a high specificity and low number of false-positives to minimize the number of patients with incorrect diagnosis of cancer. Although the risk of a malignant ovarian mass is higher in a secondary care setting and even more in a tertiary care setting, a biomarker used for diagnosis preferably has a high specificity as well. On the other hand, referral to an oncologic center for benign disease can be considered preferable above needing a second surgical procedure due to missing the diagnosis pre-operatively. Thus, ideally limited concessions should be made regarding the sensitivity of a biomarker in this setting.

A biomarker for the prediction of treatment response can select those patients who benefit from a specific treatment. A biomarker that is used for treatment monitoring and follow-up requires a correlation with disease activity and a high sensitivity in detecting disease recurrence before the onset of clinical symptoms or visible disease on radiologic imaging. Earlier detection of recurrent disease is only useful if it leads to an improved survival and quality of life. A prognostic biomarker indicates the course of a disease regardless the type of treatment.

Many biomarkers have been developed but only few of them are introduced in daily clinical practice. During this development, the before mentioned different settings are often not taken into consideration. Consequently, the purpose (screening, diagnosis, predicting treatment response and monitoring treatment, follow-up or
prognosis) of the biomarker is not well specified and it is unlikely that it performs excellent in all areas.

Human Epididymis protein 4 (HE4) has emerged as a promising serum biomarker to address some of the clinical needs in gynaecological cancer. The work presented in this thesis reports on candidate biomarker HE4 and its merits in different settings in the two most important types of gynaecological cancer: epithelial ovarian cancer (EOC) and endometrial cancer.

**Epithelial ovarian cancer**

Screening for EOC in an asymptomatic population would be the preferred option to improve the mortality rate of EOC by diagnosing patients with early stage disease. The last years, a lot of effort has been put to finding an optimal screening approach, for example with CA125.\(^3\),\(^4\) However, CA125 is a serum biomarker with a limited sensitivity. In literature, HE4 has been shown to have a similar sensitivity compared to CA125, but this has only been evaluated in a hospital population.\(^5\) Therefore, it was unclear if serum HE4 would be beneficial as a screening method in an asymptomatic high risk population. We showed that serum HE4 lacks sensitivity as a biomarker to detect early stage EOC in a population with a BRCA1 or BRCA2 gene mutation. This is not necessarily a failure of HE4, but can also be due to the quick development of disease without apparent premalignant state. Because HE4 is not useful in high risk patients it is highly unlikely that serum HE4 will be a candidate screening method in the general population.

Another approach, attractive as non-invasive and easy applicable screening test, could be a urinary test that detects HE4 protein. HE4 is secreted by the kidneys and thus detectable in urine. We found significant differences between urinary HE4 levels in healthy women, patients with benign ovarian disease and patients with EOC. HE4/creatinine ratios differentiate healthy women from those with EOC with an AUC of 0.92 (95% CI 0.79-1.00), but validation studies in a large population cohort should first be performed to calculate its sensitivity and specificity in a primary care setting. After validation, in the future a urinary HE4 stick could be developed for this purpose.

For diagnostic purposes, both HE4 and CA125 can be used since they show a similar sensitivity in differentiating between a benign and malignant ovarian mass, especially in a primary care setting, where ultrasound is not available. In other countries, HE4 is in this regard often used in a prediction model combined with CA125, called the Risk of Malignancy Algorithm (ROMA).\(^6\),\(^7\) Our results however, do not favor ROMA above HE4 alone although neither have been investigated in a primary care setting yet. Because of the higher specificity the use of HE4 is preferable over CA125 or ROMA.
The treatment of EOC in the Netherlands is centralised and warrants an accurate method in a secondary care setting to distinguish a benign ovarian mass from EOC in order to select patients that need to be referred to an oncologic center for staging surgery. This could reduce morbidity in patients with EOC because less staging surgeries in a second procedure are needed if they are correctly diagnosed preoperatively. This would also reduce the number of surgical procedures with corresponding complication risks and unnecessary testing, hospital visits and extra costs. HE4 is the preferable test in this setting and this diagnostic dilemma because of its high specificity without compromising on the sensitivity. Since ultrasound is available in a secondary care setting, it feels logical to include this in a prediction model for further evaluation of an ovarian mass. Ultrasound based models are less objective than biomarkers and require a certain amount of expertise. Information on morphological ultrasound features could complement the diagnostic process. As we reported in chapter 4 the Risk of Malignancy Index (RMI) incorrectly predicts EOC in 40% of patients, also because of its dependence upon serum CA125 levels. This limits its utility, especially in women of reproductive age who can have false-positive serum CA125 levels in benign conditions such as endometriosis. A two-step approach in which multiple tests are combined depending on their performance in different clinical settings, will possibly improve the diagnostic process of patients with an ovarian mass. This will be investigated in a future trial: HE4 Prediction (chapter 5). Other ultrasound models as developed by the International Ovarian Tumour Analysis (IOTA) collaboration group seem to outperform RMI but a comparison of these models in a RCT has never been done. This will be the aim of the currently developed IMPACT trial (IOTA and RMI Model to Predict malignancy of Ovarian Tumours).

Once a patient is referred to a tertiary care setting because of a high risk of ovarian cancer, it can be beneficial for surgical planning to estimate the risk of malignancy, the extent of disease and the chance of ovarian metastases of other malignancies. A combination of serum CA125 and CEA improves the differentiation of EOC from ovarian metastasis compared to CA125 alone, but still leaves a proportional part misclassified. Gene expression studies showed that expression of the HE4 gene and protein are more abundant in ovarian carcinomas than in other carcinomas. We showed that a combination of serum HE4 and CEA differentiates EOC from ovarian metastases of gastrointestinal origin with high accuracy. The relatively lower serum HE4 concentration in ovarian metastasis of mucinous type, is in line with previous research showing a preference of HE4 expression and secretion in serous and endometrioid EOC over mucinous EOC. This strengthens the idea that mucinous ovarian cancer (MOC) is probably more related to mucinous adenocarcinomas of gastrointestinal origin than serous type EOC, a consequence of different genetic mutation profiles. In the diagnostic work up
of patients with an ovarian mass, this could theoretically be a problem as mucinous ovarian cancers can be misclassified. However, the incidence of mucinous ovarian cancers is low and the use of staging procedures in low grade mucinous ovarian cancers is debatable. This type of EOC is often diagnosed when the disease is still limited to the ovary. Lymph node metastases rarely occur in patients with MOC, especially in low stage and low grade disease. (18) Preoperative selection of this subgroup of patients with EOC and referral to tertiary care setting for further treatment might therefore be less relevant.

Next to serum biomarkers, immunocytochemistry markers have a prominent role in the preoperative diagnosis of intra-abdominal tumours presenting with ascites. In advanced stage EOC, ascites is often easier to obtain than a histological biopsy but more difficult to interpret. HE4 exhibits the beneficial characteristic that it cannot only serve as a serum biomarker but also as immunocytochemistry marker in ascites. Moreover, HE4 staining is highly specific to tumour cells in ascites and cannot be confused with mesothelial cells that can have similar morphologic features. Our results support the use of HE4 as immunocytochemistry marker in a panel with PAX8, to facilitate the preoperative determination of tumour origin in case of malignant ascites. This is of clinical importance in light of the increase in number of interval debulking surgeries at the expense of primary debulking in the last couple of years. (19) The consequence of this different approach in the treatment of high stage EOC is, that neoadjuvant chemotherapy is often started based on a cytological diagnosis only.

A role of serum HE4 monitoring of treatment and follow-up will become essential as soon as adequate treatment for recurrent disease is available. The majority of patients with advanced stage EOC will suffer from disease recurrence with a dismal prognosis. But early detection by biomarkers has not proven to be beneficial for overall survival and quality of life. (23)

A tissue biopsy could solve the problems regarding the preoperative diagnosis of an ovarian mass and could confirm a malignancy and the type of malignancy. However, this is an invasive method and may cause spill of tumour cells in the abdomen and may technically not always be possible. So called ‘liquid biopsies’ overcome the problems of tissue biopsies: they are quick, minimally invasive and give the opportunity to assess tumour genomes before and during treatment. (20,21) The idea of liquid biopsies is based on the fact that tumour cells or fragments thereof enter the blood stream. Liquid biopsies include tumour educated platelets (TEP), cell-free circulating tumour DNA (ctDNA) and circulating tumour cells (CTC). mRNA sequencing of TEP accurately differentiated healthy individuals from cancer patients with 96% sensitivity and can thereby offer valuable diagnostic information for all cancer patients, including those with localized disease. (22) Detectable levels of ctDNA were found in most patients (>80%) with metastatic cancer, including EOC.
(20) The fraction of detectable ctDNA was lower in patients with localized disease but this has not been evaluated in EOC. It has been shown that tumours with 50,000 cells release enough fragments to be picked up in the circulation. This implicates that tumours visible on ultrasound should be detectable by ctDNA. (21) Possibly, information on genetic alterations acquired by liquid biopsies can also be used to diagnose different histological subtypes of EOC (serous vs mucinous) and to select patients that need staging procedures. (20)

Liquid biopsies are promising and might offer new options in the diagnosis of ovarian cancer. However, their clinical utility and financial burden to the health care system have to be further addressed. A proposal for a prospective study to evaluate the diagnostic accuracy of liquid biopsies as molecular biomarkers in the diagnosis of patients with an ovarian mass and compare this with other non-molecular biomarkers as HE4 and RMI, is therefore currently under review for funding.

**Endometrial cancer**

A biomarker that supports the diagnosis of endometrial cancer (EC) is not as urgently needed as for EOC. In a primary care setting patients are often identified by the early onset of symptoms such as postmenopausal bleeding. They are immediately referred to a gynaecologist to measure endometrial thickness and if necessary, to obtain a pathological diagnosis by endometrial biopsy. With endometrial biopsy, high sensitivity and specificity results for the diagnosis of EC can be achieved (98-100% and 99-100%, respectively). Even for premalignant endometrial lesions (complex atypical hyperplasia) a sensitivity of 81% is obtained.(26) It is therefore highly unlikely that HE4 could improve this diagnostic test.

However, this does not mean that there is no need for a biomarker in EC at all. A biomarker that predicts the extent of disease or prognosis can be useful. Also in patients who prefer to preserve their fertility with conservative treatment this prediction is important. EC is divided into two groups.(27) The most common type is the low-grade endometrioid type EC which generally has a good prognosis. In contrast, the less common non-endometrioid, high grade, TP53-mutated tumours are associated with a significantly worse prognosis. Histologic subtype and tumour grade can be estimated in a preoperatively obtained endometrial biopsy, but may change after histological and immunohistochemical investigation of the uterus. (28,29) Other known pathological prognostic factors can only be determined after removal of the uterus, adnexa and sometimes lymph nodes. Several biomarkers have been described as prognostic factors in EC: e.g DNA ploidy, oncogenes, tumour suppressor genes, mismatch repair genes, microsatellite instability.(30) None of them has achieved clinical relevance so far. Various studies including ours (chapter 10) have shown a correlation of preoperatively obtained serum HE4 concentrations with known pathological prognostic factors in EC: stage of disease,
deep myometrial invasion, cervical invasion and LVS1. (31–33) A combination of serum CA125 and HE4, for example in a similar algorithm as ROMA, possibly increases the prognostic accuracy of each marker alone but this has to be further evaluated. (34) Whether serum HE4 is superior to radiologic imaging techniques (CT-scan, MRI scan) in determining the presence of cervical invasion and extent of myometrial invasion has not been investigated yet. A two-step approach where a high grade tumour in endometrial biopsy or an elevated serum HE4 concentration prompts doing a MRI scan to evaluate myometrial invasion and lymph node metastases, can be considered. A low grade tumour in combination with a normal HE4 concentration almost certainly rules out deep myometrial invasion. This method reduces the number of unnecessary and costly MRI scans.

In the near future, subdivision of EC tumours into risk groups will probably be based on molecular subgroups: POLE mutation, microsatellite instability, copy-number low and copy-number high. (35) Genetic analysis has emphasized the heterogeneity of EC and this is not fully covered by the current dualistic histological model and tumour grade. A new classification leading to a more individualized treatment plan should be developed. Our results support further research to the use of HE4 as biomarker in EC patients and including this in the treatment plan.

Tailored treatment is the adagium of all research nowadays. We are convinced that HE4 will claim its place in treatment protocols of ovarian and endometrial cancer.
References


