Current clinical application of serum biomarkers to detect and monitor ovarian cancer – update

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Abstract

In recent decades many potential serum biomarkers have been assessed in the diagnosis of ovarian cancer. Except cancer antigen 125 (CA125) and human epididymis protein 4 (HE4), none of them have been applied to everyday clinical practice. Based on extensive scientific evidence, CA125 combined with HE4 to form the risk of ovarian malignancy algorithm (ROMA), have become widespread in clinical practice in the evaluation of adnexal masses. Early ovarian cancer is often asymptomatic, so it remains challenging to develop even more effective methods for early diagnosis and screening. Among others, OVA1 is tested as a potential tool to improve the stratification of the risk of ovarian cancer. Also, a lot of effort is being made to develop suitable methods to monitor ovarian cancer patients. Serum CA125 already plays an established role in monitoring the treatment (except targeted therapies) and relapse setting in ovarian cancer patients, with a more limited role in subtypes other than in high-grade serous carcinoma, and always in correlation with imaging and clinical assessment. Human epididymis protein 4 (as well as circulating tumour DNA – ctDNA) is not recommended for monitoring at that timepoint, although encouraging newly published studies might influence their role in the future.

Key words: ovarian cancer, biomarkers, CA125, HE4, ROMA, OVA1.

Introduction

Approximately 20% of women are expected to suffer from an adnexal mass at least once in their lifetime [1]. About half of them undergo surgery for this reason [2]. The population risk of ovarian cancer is about 1–1.5%, which means that the majority of adnexal tumours are benign and can be operated by general gynaecologists [3]. However, many benefits have been proven for patients with ovarian cancer operated by oncological gynaecologists compared with general gynaecologists and surgeons (more accurate staging, more precise cytoreductive surgery, fewer complications, higher percentage of 5-year survival) [4–6]. Therefore, women with suspected ovarian tumours should be directed to centres specialized in oncological gynaecology.

On that basis, new methods of selecting patients at a high risk of ovarian malignancy are investigated. Nowadays, physical bimanual examination, gynaecological ultrasound imaging, and serum biomarkers are used to assess adnexal masses. Some authors question the utility of ultrasound because of its subjectivity and dependence on sonographer's experience [7]. Consequently, the importance of serum markers in the diagnosis of ovarian cancer is growing as they become more objective and comparable.

In recent years, a wide spectrum of cytokines, growth factors, adhesion molecules, proteases, hormones, co-agulation factors, acute phase reactants, and apoptosis factors have been investigated as potential single serum biomarkers and in multimarker panels in diagnosing ovarian cancer, but only cancer antigen (CA125) and HE4 have been applied to everyday clinical practice.

Serum biomarkers in preoperative diagnosing of ovarian tumour

Cancer antigen 125

Cancer antigen 125 is a glycoprotein, encoded by *MUC16* gene on chromosome 19. Its upper limit of normal value is set at 35 UI/ml. Expression of CA125 is elevated in 85% of serous, 65% of endometrioid, 40% of clear-cell, 36% of undifferentiated, and 12% of mucinous ovarian cancers [8]. For the last 3 decades it has been the most widespread biomarker of ovarian cancer. The utility of CA125 in the diagnosis of ovarian cancer has been evaluated in many studies.

Serum levels of CA125 are within the normal limits in at least 20% of patients with ovarian cancer and in about half of patients in its early stages, which consid-

erably reduces the sensitivity of this marker. Moreover, CA125 concentrations are elevated in many non-malignant conditions, which significantly affect its specificity. The most important cause of false-positive results of CA125 is endometriosis (in about two-thirds of patients with endometriotic cysts, CA125 levels exceed the normal range) [8, 9]. Cancer antigen 125 levels are also raised in patients with other gynaecological diseases (such as myomas of the uterus, benign and borderline ovarian tumours), many non-gynaecological illnesses (e.g. hepatic cirrhosis, congenital heart defects), during pregnancy, and in 1–5% of healthy women [8, 10–12]. Because of the aforementioned limitations of CA125 protein, many studies have been conducted to improve the ovarian cancer diagnostic protocol.

Attempts have been made to combine CA125 with ultrasound imaging, resulting in the development of many diagnostic algorithms [13]. One of them, the risk of malignancy index (RMI), has been applied in clinical practice. Four variations of RMI have been developed, the first of which (RMI I) is the most efficient [14–17]. It is calculated with the use of the following formula:

RMI = U × M × CA125,

where: U – ultrasound image (1 point for each of the features: solid, multilocular, bilateral tumour, ascites, intra-abdominal metastases); U = 0 (0 points), U = 1 (1 point), U = 3 (2-5 points); M – menopausal status; M = 1 (premenopausal), M = 3 (postmenopausal); CA125 – serum CA125 concentration (U/ml).

RMI values > 200 qualify patients to the group of high risk of malignancy, reaching the sensitivity of 78% at the specificity of 87% [13]. The risk of malignancy index has become a practical diagnostic tool, which is still used in the diagnosing of ovarian tumours.

Human epididymis protein 4

Human epididymis protein 4 (HE4) is a glycoprotein encoded by the *WFDC2* gene (chromosome 20) [18]. Presumably, it takes part in the immune response, but its role has not been precisely specified [19, 20]. It is present in the epithelium of fallopian tubes, endometrium, and endocervical glands, but not in ovarian surface epithelium. Expression of HE4 has also been noted in the epithelium of the respiratory tract (especially the trachea), renal convoluted tubules, and salivary glands [21, 22]. An elevated expression of HE4 is observed in 93–100% of serous, 80–100% of endometrioid, and 50–83% of clear-cell carcinomas of the ovary, while it is absent in mucinous ovarian cancer [21, 22].

In preliminary studies HE4 has proved to be more sensitive (at preset specificity) than any other ovarian cancer marker, including CA125. It was elevated in over 50% of ovarian cancer patients with CA125 levels within normal limits. A combination of both CA125 and HE4 had higher sensitivity than any other marker combination, and HE4 achieved the highest sensitivity among all tested proteins in the diagnosis of early ovarian cancer [1].

HE4 proved to be less frequently elevated than CA125 in benign ovarian tumours, both in pre- and postmenopausal women. Most of all, HE4 levels exceed normal ranges much less frequently than CA125 in cases of endometriosis (3% vs. 67%) [9]. These observations have been confirmed by other authors [23, 24]. Serum concentrations of HE4 are also more rarely increased than CA125 in serous cysts, teratomas, fibromas, and inflammatory lesions. However, serum levels of both CA125 and HE4 do not show statistically relevant differences in patients with mucinous cysts [9]. Because HE4 levels are not affected by many benign diseases that increase concentrations of CA125, HE4 is a valuable complement in distinguishing malignant from benign ovarian tumours.

In women with benign non-gynaecological diseases, serum concentrations of HE4 are increased less frequently than CA125 [25–27]. According to Escudero *et al.*, levels of HE4 and CA125 in this group of patients exceeded the reference values in 12.3% and 37%, respectively [25]. Even half of the false-positive results of HE4 can be observed in the course of renal failure [25]. A statistically significant increase in HE4 levels was demonstrated in the course of chronic kidney disease, proportional to the decrease in glomerular filtration rate [28]. An increase in HE4 protein levels was also found in people with heart failure, proportional to its severity [29]. The increase in HE4 levels was also observed among patients with liver and lung diseases [25]. Smoking is associated with a 21–29% increase in levels of this biomarker [26, 30]. In one of the studies, a decrease in HE4 concentrations was observed with an increase of body mass index [26].

In contrast to CA125, HE4 concentrations were reported by Moore *et al.* to be lower in pregnant women when compared with their premenopausal counterparts [31]. Levels of HE4 do not differ significantly between trimesters. Only between the second and third trimester of pregnancy, a slight statistically insignificant increase in the HE4 concentrations was reported [31]. On the other hand, Park *et al.* observed a slight, but statistically significant, increase in HE4 concentrations during pregnancy [27]. However, the authors of the aforementioned study note that pregnancy has a smaller influence on HE4 than CA125 levels. According to Gucer *et al.*, pregnancy and its course do not significantly affect the serum concentrations of HE4 [32]. Therefore, it seems that the HE4 protein may be a useful tool in the diagnosis of adnexal lesions in pregnant women, although the available data come from studies on a limited group of patients. As a result, unfortunately, their results are inconclusive and should be assessed with caution.

It has been proven that hormonal therapy does not affect HE4 serum levels. Therefore, oral contraception,

treatment of menstrual disorders and endometriosis do not need to be aborted for HE4 testing. The influence of hormonal replacement therapy on HE4 concentrations has not been well defined yet [33].

HE4 levels do not alter during the menstrual cycle, so they can be determined regardless of its phase [33]. Among healthy women, concentrations of HE4 increase with age (starting from the age of 40 years), especially in the 8th and 9th decade of life. This fact ought to be taken into account when the serum of elderly women is tested, because elevated levels of HE4 may lead to false-positive results in this group of patients [31].

Unfortunately, the normal limits for HE4 have not been well established yet. Depending on the study, normal ranges vary from 70 to 150 pM [1, 34]. Moore et al. determined normal values separately for patients before and after menopause (89.1 pM and 128.0pM, respectively) [31].

Risk of ovarian malignancy algorithm

Based on the encouraging results of HE4 in the diagnosis of ovarian cancer, especially in combination with CA125, Moore et al. developed the risk of ovarian malignancy algorithm (ROMA) [7]. It utilizes serum concentrations of both CA125 and HE4, which are substituted to the mathematical formula, elaborated separately for pre- and postmenopausal patients.

Premenopausal patients

$PI = -12.0 + 2.38 \times LN (HE4) + 0.0626 \times LN (CA125)$

Postmenopausal patients

$PI = -8.09 + 1.04 \times LN (HE4) + 0.732 \times LN (CA125)$

$ROMA (\%) = \exp (PI)/[1 + \exp (PI)] \times 100\%$

According to the cut-off values established by the authors, ROMA value > 13.1% in pre- and > 27.7% for postmenopausal women qualified them to a group with a high risk of malignancy of ovarian tumour. Cut-off values differ slightly depending on the manufacturer of the diagnostic kit.

The authors of this algorithm revealed its sensitivity at the level of 93.8% (88.9% for pre- and 94.6% for postmenopausal women) at the specificity of 75% in the diagnosis of epithelial ovarian cancer [7]. When compared with RMI, ROMA demonstrated higher sensitivity in the diagnosis of ovarian cancer (94.3% vs. 84.6% at 75% specificity). When early stages of ovarian cancer (FIGOI/II) were concerned, the difference between these 2 tests was even more remarkable (85.3% for ROMA vs. 64.7% for RMI at 75% specificity) [35].

Many studies evaluating the utility of HE4 and ROMA in the diagnosis of ovarian cancer have been published. Although most of them confirm the effectiveness of both methods, according to authors of some studies, adding these methods to the diagnostic protocol is not clearly justified [36–39].

Van Gorp et al. proved that not only is ROMA worse than RMI, but the subjective assessment of adnexa by an experienced sonographer exceeds both models [37]. Kaijser et al. did not show any benefit from HE4 measurement and ROMA quantification in patients with pelvic tumours after previous ultrasonographic assessment [38]. Attempts to use HE4 protein concentrations instead of CA125 to calculate RMI did not bring a statistically significant improvement in the effectiveness of the index in the preoperative diagnosis of ovarian cancer [39].

Despite some controversy over HE4 and ROMA, most of the available meta-analyses confirm their utility [40–42]. According to Lin et al., ROMA is more sensitive than CA125 and HE4 in the diagnosis of ovarian cancer (sensitivity of 87% at specificity of 82%). Surprisingly, CA125 reveals even higher sensitivity than HE4 (80% vs. 74%). Most importantly, HE4 proved to be the most specific of all 3 methods (specificity of 87% for HE4 vs. 82% for ROMA vs.76% for CA125) [42]. However, due to the heterogeneity of studies, the results of meta-analyses should be treated with caution [40–42].

Nevertheless, HE4 and ROMA have already established their role in the preoperative diagnosis of ovarian tumours.

Ovarian cancer test

In 2009, the U.S. Food and Drug Administration approved for clinical use a new test, OVA1 [43]. It evaluates serum concentrations of 5 markers. Two of them are upregulated (CA125 II, β-microglobulin) and 3 are downregulated (apolipoprotein A1, prealbumin, transferrin) in patients with ovarian cancer. Serum levels are compiled with the use of a computer program – OvaCalc® – giving a result as a number between 0 and 10. Values ≥ 5.0 in premenopausal and ≥ 4.4 in postmenopausal women qualify patients to the high-risk group. Patients with positive result of the OVA1 test should be referred to the oncological gynaecologist [44].

Results of a multicentre study (OVA500 Study) showed the sensitivity of the OVA1 test in the diagnosis of ovarian cancer at the level of 96% (91% in FIGO I/II) and specificity of 51%. Also, a high negative predictive value of the OVA1 test is noteworthy [45].

Few studies evaluating the utility of the OVA1 test have been published. However, available publications confirm to some extent the results of the OVA500 study. According to one of the authors, OVA1 reveals sensitivity and specificity at the level of 96% and 28% in postmenopausal and 85% and 40% in premenopausal women, respectively [46]. In one study, OVA1 qualified to the high-risk group 76% of patients with malignant adnexal tumours, who had serum CA125 levels within the normal limits [47]. Some reports also confirm high sensitivity of the OVA1 test in the diagnosis of early

ovarian cancer [48, 49]. Although Dunton *et al.* prove some utility of OVA1 in management of tumours with low-risk malignancy, this algorithm has not yet been implemented into everyday practice [50].

Serum biomarkers in the monitoring of ovarian cancer

According to the ESMO-ESGO recommendations published in 2019, serum CA125 is useful in clinical practice but only in combination with clinical and radiological assessment. It is important to emphasize that the CA125 value is well studied in high-grade serous carcinoma, but in patients with low-grade serous, endometrioid, mucinous, or clear cell ovarian cancer surveillance should not be based on CA125 as an equally reliable marker [51]. Moreover, we do not have enough data to support CA125 values in patients treated with targeted therapies, such as bevacizumab or olaparib. The prognostic or predictive for relapse meaning of HE-4 changes was studied in combination with CA125, and radiological and clinical, assessment but the studies led to conflicting results, and finally HE-4 was not recommended in routine practice in response or progression evaluation.

Recently published study by Potenza *et al.* aimed to assess the ability of CA125 and HE-4 to identify patients at higher risk of non-optimal response and of recurrence of disease during systemic therapy of 78 epithelial ovarian cancer patients after debulking surgery or interval debulking surgery after neoadjuvant systemic therapy. Both CA125 and HE-4 were measured at baseline and at each chemotherapy cycle. Computed tomographies were performed to confirm objective response. The authors found that in all cases of good response to chemotherapy, CA125 and HE-4 decreased to normal values after the fourth chemotherapy cycle. HE-4 had a more rapid decrease rate during chemotherapy than CA125, which had a delay of 21 days. Moreover HE-4 was re-detected faster than CA125 in patients who did not have a good chemotherapy response [52].

A study published in 2021 assessed the HE-4 and CA125 early clearance prognostic value for platinum sensitivity, 2-year progression-free survival (PFS), PFS, and overall survival (OS) in 89 patients with epithelial ovarian cancer after initial staging surgery or optimal cytoreduction, who received 6–8 cycles of adjuvant chemotherapy (16 platinum-resistant and 73 platinum-sensitive). Human epididymis protein 4 and CA125 clearance was defined as a 90% decrease from baseline value or reduction to normal. The study demonstrated that PFS and OS were associated with HE-4 clearance after the 3rd course of chemotherapy ($p < 0.0001$ for both), and CA125 clearance after the 1st chemotherapy cycle ($p < 0.0001$ for both), confirmed in a multivariate Cox regression analysis as independent prognostic factors. Thus, the study confirms that HE-4 and CA125 monitoring during first-line chemotherapy is useful for prognosis in terms of platinum sensitivity, PFS, and OS in epithelial ovarian cancer patients [53].

Japanese researchers conducted a study that aimed to evaluate droplet digital PCR as a method of detection of relapse in 11 ovarian cancer patients [54]. They analysed the relationship of the onset of recurrence, recurrent tumour size, and the duration of PFS with CA125 and circulating tumour DNA (ctDNA) analysis for individual mutations detected in high-grade serous and clear-cell ovarian carcinoma patients who were at high risk of relapse. As a result, mutated ctDNA fragments in plasma were detected in all 6 patients with recurrence during follow-up, earlier than CA125 changes (49 days and 7 days before imaging showed relapse, respectively: $p < 0.05$). No ctDNA was detected in recurrence-free patients. Thus, the method is highly sensitive and specific, but probably not useful in clinical practice as individual molecular diagnosis and repeated detection of patient-specific mutation pattern is difficult and expensive. Moreover, the ESMO-ESGO recommendations clearly indicate that ctDNA is not a tool to assess response or relapse [51].

Conclusions

Taken together, clinical examination, ultrasonography, and serum markers (ROMA) accurately select patients with ovarian tumours of a high risk of malignancy, which facilitates their referral to centres specializing in oncological gynaecology. Serum CA125 plays an established role in monitoring the treatment (except targeted therapies) and relapse setting in ovarian cancer patients, with a more limited role in subtypes other than high-grade serous carcinoma, and always in correlation with imaging and clinical assessment. HE-4 and ctDNA are not recommended for monitoring at that timepoint, although encouraging newly published studies might influence their role in the future.

Disclosure

The authors report no conflict of interest.

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