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Clinical Use of Cancer Biomarkers in Epithelial Ovarian Cancer

Updated Guidelines From the European Group on Tumor Markers

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Objective: To present an update of the European Group on Tumor Markers guidelines for serum markers in epithelial ovarian cancer.

Methods: Systematic literature survey from 2008 to 2013. The articles were evaluated by level of evidence and strength of recommendation.

Results: Because of its low sensitivity (50–62% for early stage epithelial ovarian cancer) and limited specificity (94–98.5%), cancer antigen (CA) 125 (CA125) is not recommended as a screening test in asymptomatic women. The Risk of Malignancy Index, which includes CA125, transvaginal ultrasound, and menopausal status, is recommended for the differential diagnosis of a pelvic mass. Because human epididymis protein 4 has been reported to have superior specificity to CA125, especially in premenopausal women, it may be considered either alone or as part of the risk of ovarian malignancy algorithm, in the differential diagnosis of pelvic masses, especially in such women. CA125 should be used to monitor response to first-line chemotherapy using the previously published criteria of the Gynecological Cancer Intergroup, that is, at least a 50% reduction of a pretreatment sample of 70 kU/L or greater. The value of CA125 in posttherapy surveillance is less clear. Although a prospective randomized trial concluded that early administration of chemotherapy based on increasing CA125 levels had no effect on survival, European Group on Tumor Markers state that monitoring with CA125 in this situation should occur, especially if the patient is a candidate for secondary cytoreductive surgery.

Conclusions: At present, CA125 remains the most important biomarker for epithelial ovarian cancer, excluding tumors of mucinous origin.

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Cancer antigen 125 (CA125) is currently the only serological biomarker in routine use for the management of patients with epithelial ovarian/fallopian tube or primary serous peritoneal cancer.[1] The reference interval for CA125 is 35 kU/L or less.[2] Elevated concentrations can occur in healthy premenopausal women during menses, in pregnancy, and in nonmalignant gynecologic diseases, such as ovarian cysts, endometriosis, adenomyosis, and uterine leiomyomas. High serum concentrations may also occur in several nonmalignant nongynecological diseases, such as peritoneal, pleural, and musculoskeletal inflammatory disorders as well as pelvic inflammatory disease, liver, and renal as well as cardiac disease (Fig. 1). Additionally, elevated concentrations can occur in most types of advanced adenocarcinomas, including breast, colorectal, pancreas, lung, endometrium, and cervix (Fig. 2).[4–6] In women with epithelial ovarian cancer, approximately 80% have concentrations above 35 kU/L, with elevations in 50% to 60% of patients with clinical stage I disease, 80% to 90% in stage II, and greater than 90% in stages III to IV.[4,5] However, the frequency of elevated concentrations is highest in patients with serous epithelial ovarian cancer followed by endometrioid and clear cell types.[4,5] The CA125 is not expressed in pure mucinous tumors and is not useful among patients with this histological type of epithelial ovarian cancer.[4,7,8] Carcinoembryonic antigen or CA19.9 may be better markers in these patients.[7,9]

METHODS

Literature was searched in the Medline Database, using the following criteria: human epididymis protein 4 (HE4), ovarian carcinoma, CA125; and screening or diagnosis or prognosis or monitoring; and ovarian carcinoma or HE4 or human epididymis protein 4, or CA125. Filters are publication dates from January 1, 2008, to December 31, 2013. All of the titles were generated by the search, and the abstracts were reviewed for relevance, after which the full articles were obtained for those selected. The articles were evaluated by level of evidence (LOE) and strength of recommendation (SOR) according to the classifications provided in Tables 1 and 2, respectively.[10,11] Earlier guideline articles and their references were also searched.[4,8] The results of the literature search were structured according to the types of marker utility as presented in Table 3.

RESULTS

CA125 Screening

The Prostate, Lung, Colorectal, and Ovarian Cancer trial in the United States was a randomized controlled trial in which 78,216 women aged 55 to 74 years were included between 1993 and 2001. There was no evidence of a shift to early-stage disease associated with screening using CA125 and transvaginal ultrasound. Furthermore, ovarian cancer mortality was equivalent in both groups.[12] An earlier multicenter randomized controlled trial was conducted in Japan between 1985 and 1999, in which postmenopausal women were assigned to either a screening group (n = 41,688) or a control group (n = 40,799). The screening group was assigned to pelvic examination, transvaginal ultrasound, and CA125. No tests were applied in women allocated to the control group. The study showed a decrease in stage at detection; however, analysis of mortality in the screening and control group has not yet been reported.[13] In a single-arm prospective study, the University of Kentucky Ovarian Cancer Screening Trial conducted from 1987 to 2011, 37,293 women were screened annually with ultrasound and CA125.[14] Eligibility criteria included all asymptomatic women aged 50 years or older and women aged 25 years or older with a documented family history of ovarian cancer. Although there was no randomization against a control group, a historic control group was available, consisting of 380 patients diagnosed with ovarian cancer during the study period. The trial suggested a decrease in stage at detection as well as a survival benefit.[14] Another single-arm, prospective multicenter study, also from the United States, investigated the utility of a 2-stage ovarian cancer screening strategy using a risk of ovarian cancer algorithm among 4051 postmenopausal women. Rising concentrations of CA125 above cutoff 35 kU/L prompted ultrasound investigation. The study showed a specificity and positive predictive value of 99.9% and 40%, respectively.[15] The United Kingdom Collaborative Trial of Ovarian Cancer Screening is

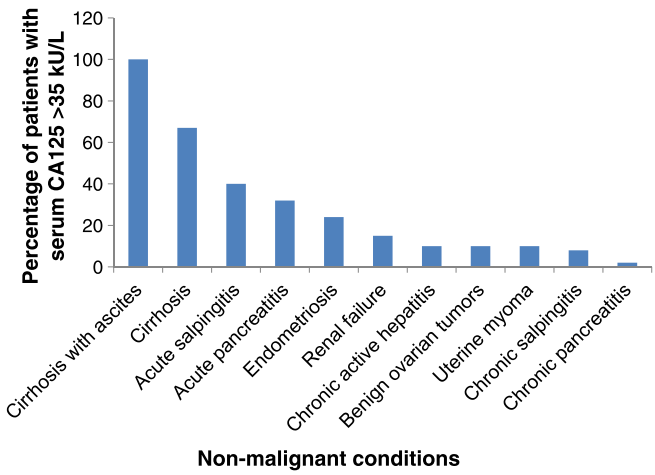

FIGURE 1. Nonmalignant conditions causing elevated CA125 concentrations.[3]

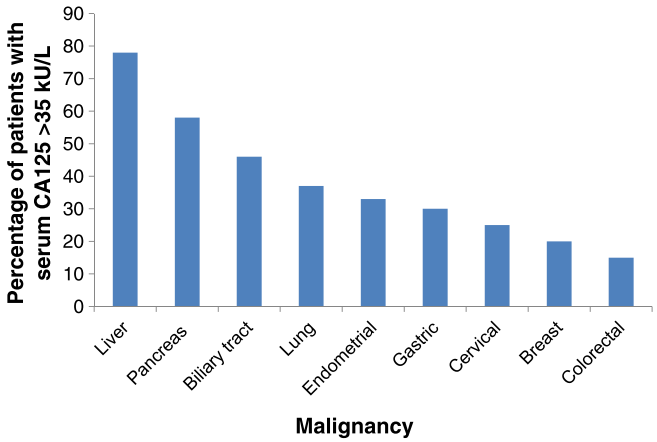

FIGURE 2. Frequency of elevated CA125 concentrations in different malignancies.[3]

ongoing.[16] From 2001 to 2005, 202,638 postmenopausal women, aged 50 to 74 years were randomly assigned to annual transvaginal ultrasound alone (N = 50,639) or annual CA125 with transvaginal ultrasound performed at rising CA125 concentrations (N = 50,640) or no investigative procedures (N = 101,359). The trial is expected to conclude in 2015, and the effect of diagnosis at early stage of disease on ovarian cancer mortality awaits analysis of these data.[16]

At present, the conclusion from these major trials is that owing to limitations of CA125 sensitivity and specificity, its use among asymptomatic women outside the context of a clinical trial cannot be recommended for general population screening (Table 4).[4] However, CA125, in combination with transvaginal ultrasound, may have a role in early detection of ovarian cancer in women with hereditary alterations in the BRCA1 and BRCA2 tumor suppressor genes, where the lifetime risk of developing ovarian cancer is approximately 40% for BRCA1 carriers and 18% for BRCA2 carriers.[22] However, there is as yet no evidence that ovarian cancer screening results in a stage shift to earlier stage disease, or that it reduces morbidity or mortality from ovarian cancer. The best prevention in these women is bilateral salpingooophorectomy.[23–25]

European Group on Tumor Markers Statement

• Screening for ovarian cancer based on CA125 is not recommended among asymptomatic women due to lack of sensitivity both for stage I disease and for mucinous epithelial ovarian tumors. CA125 also lacks specificity, especially for premenopausal women.

(LOE I, SOR B)

Differential Diagnosis

Postmenopausal women with CA125 concentrations greater than 35 kU/L should be considered for transvaginal ultrasound examination as well as a computed tomography scan. The CA125 concentrations greater than 95 kU/L has been reported to discriminate malignant from nonmalignant pelvic masses with a positive predictive value of 95%.[8] For premenopausal women, the American College of Obstetrics and Gynecologists suggested that patients with a pelvic mass and CA125 concentrations greater than 200 kU/L should be referred to a gynecologist for consultation.[26]

Algorithms to calculate the Risk of Malignancy Index (RMI) have been developed by Jacobs et al[27] and by Tingulstad et al[28] as RMI 1 and RMI 2, respectively. Both RMI scoring systems are the product of ultrasound score × menopausal score × CA125 concentration in kU/L (Table 5). The difference between the RMI 1 and the RMI 2 scores is the number of ultrasound findings considered. Three studies have compared the 2 RMI systems using score values above 200 to indicate malignancy. The validity of the RMI 1 and the RMI 2 scores was similar.[28–30] Another algorithm was developed and validated in co-operation with a number of European centers specialized in ultrasound of the pelvis (International Ovarian Tumor Analysis group). The algorithm challenged the suggestion that the CA125 concentration added to ultrasound in distinguishing nonmalignant from malignant ovarian masses.[31] In the hands of these expert centers, the ultrasound criteria only performed better than the RMI 1.[32] The same group has, however, reintroduced CA125 in the latest version of their algorithm,

TABLE 1. Level of Evidence Used to Grade the Presented EGTM Guidelines[10]

LOE	Criteria
I	Evidence of a single high powered, prospective, controlled study that is specifically designed to test the index marker, or evidence from a meta-analysis, pooled analysis, or overview of level II or III studies.
II	Evidence from a study in which marker data are determined in relationship to a prospective therapeutic trial that is performed to test therapeutic hypothesis but not specifically designed to test marker utility.
III	Evidence from large prospective studies.
IV	Evidence from small retrospective studies.
V	Evidence from small pilot studies.
Expert opinion	Formal consensus of sub-committee members.

TABLE 2. Strength of Recommendation Used to Grade the Presented EGTM Guidelines[11]

SOR	Criteria
High (A)	Further research is very unlikely to change the confidence in the estimate
Moderate (B)	Further research is likely to change the estimate
Low (C)	Further research is very likely to change the estimate
Very low (D)	Estimate is very uncertain

Assessment of Different NEoplasias in the adneXa.[33] The National Institute for Health and Care Excellence has introduced guidelines for early detection of ovarian cancer in symptomatic women for use by general practitioners.[34] The potential advantages and disadvantages of the guidelines have been discussed with focus on the use of CA125 among premenopausal women where its increasing use may lead to waste of health care resources.[35] The recommendations from different scientific societies are provided in Table 4.

European Group on Tumor Markers Statement

• The RMI calculated either as RMI 1 or as RMI 2 is recommended for differential diagnosis of non-malignant and malignant pelvic masses in postmenopausal women.
(LOE II-III, SOR B)

Prognosis

Prognosis Based on a Single Measurement

Preoperatively, the initial stage of disease is an important prognostic factor. However, it has been suggested that in patients who had a preoperative CA125 concentration greater than 65 kU/L, the 5-year survival rates in univariate and multivariate analyses were found to be significantly lower as compared to patients who had values less than 65 kU/L. For the studies including early-stage disease (IA, B, C), the initial CA125 values would be more closely related to histology (serous vs nonserous) rather than prognosis within the serous population.[36,37] Studies by Prat et al and Xu et al[38,39] based on multivariate analysis suggested that the nadir concentrations after primary treatment and follow-up may provide prognostic information in terms of overall survival (OS). However, this information needs confirmation because it is not unusual to observe transient elevations in CA125 after chemotherapy, likely reflecting tumor necrosis and release of circulating CA125. Finally, there has been no consistent effort to differentiate between patients who have primary optimal cytoreduction (which can reduce CA125 before chemotherapy) and patients selected for neoadjuvant chemotherapy with interval cytoreduction, who must rely only on chemotherapy. As such, knowledge of single-point CA125 measurements would not change ongoing primary therapy and provides only limited prognostic information. The inconsistent results from different studies may also be attributed to the unspecific use of CA125 for all epithelial ovarian cancer types.

Prognosis Based on a Change in Measurements

Markman et al[40] reported that a decrease in CA125 concentrations of 50% or greater during the initial 2 cycles of platinum-based chemotherapy was a powerful independent prognostic indicator for OS. Riedinger et al[41] reported that one third of CA125 decrease patterns observed among 130 stages IIc to IV patients receiving paclitaxel or platinum-based chemotherapy were biexponential with a half-life greater than 14 days, indicating persistent CA125 production and a poor response to chemotherapy and impaired survival. Van Altena et al[42] found that patients who achieved complete clinical remission after standard primary treatment and also reached a CA125 nadir concentration less than 5 kU/L had a significantly longer progression-free survival and OS than patients with nadir values between 6 and 35 kU/L. Overall, all investigators reported that a prolonged half-life was indicative of persistent CA125 production and was predictive of a poor response to chemotherapy.[41] The recommendations by different scientific societies are provided in Table 4.

European Group on Tumor Markers Statement

• **A** change in sequential measurements during primary treatment **is** recommended as prognostic indicator for response to treatment.
(LOE III/IV, SOR B)

TABLE 3. Utility of Cancer Biomarkers as Defined in the Presented EGTM Guidelines

Cancer Biomarkers	Definitions
Screening markers	In asymptomatic people to detect a disease or condition at an early stage
Differential diagnostic markers	In people with signs or symptoms to aid in assessing whether they have a condition
Prognostic markers	Classify patients treated with standard therapies (including no treatment if that is standard) into subgroups with distinct expected outcomes
Monitoring markers	Early informers on changing tumor burden or tumor activity
Predictive markers	Identify patients whose tumors are likely to be sensitive and/or resistant to a specific agent

Monitoring

The utilization of CA125 to monitor tumor response was initially developed for evaluation of new treatments in the setting of recurrent disease. However, the mentioned criteria may also be considered during primary therapy because CA125 is used in routine clinical practice.

Criteria to Define Decrements

Rustin et al[43] have proposed a set of definitions for CA125 decrements as at least a 50% reduction or a 75% reduction of an elevated pretreatment concentration. Patients can be evaluated with CA125 if the pretreatment concentration is at least twice the upper limit of normal.[44] The Gynecological Cancer Intergroup reached a consensus in 2011 where the criteria to evaluate decrements proposed by Rustin et al[45] were simplified and included in The Response Evaluation Criteria in Solid Tumors for use in first-line trials in ovarian cancer. A CA125 response was defined as at least a 50% reduction in CA125 levels from a pretreatment sample. The response must be confirmed and maintained for at least 28 days.[46] Another definition was suggested by Tuxen et al[47] who based interpretation of decreasing concentrations, on a statistical estimation adjusted to both analytical and biological variation of the marker. A similar methodology has been proposed to interpret biomarker changes during monitoring of patients with breast and prostate cancer.[48,49]

Criteria to Define Increments

The criteria introduced by Rustin et al[50,51] depend on the CA125 concentrations. For patients with elevated pretreatment concentrations that normalize on first-line chemotherapy, the criterion require increasing concentrations to twice the upper limit of normal (>70 kU/L). For patients with elevated pretreatment concentrations that never normalizes, the criterion was a doubling of the nadir value.[51] Tuxen et al[47,52] also suggested 2 criteria depending on whether the increment started below or above the cutoff. For an increment starting below the cutoff, the criterion was a significant increase to above the cutoff. For an increment starting above cutoff, the criterion was a significant increase from the baseline concentration.

Design of Tumor Marker Monitoring Trials

Rustin et al[53] enrolled 1442 women in complete remission after first-line platinum-based chemotherapy and a normal CA125 concentration. The majority of patients (>90%) had advanced stage ovarian cancer. They compared the outcome after the initiation of treatment of relapsed ovarian cancer based on rising CA125 levels from below cutoff (≤35 kU/L) to twice the upper limit of normal (>70 kU/L) versus the initiation of treatment commencing at clinical relapse. The patients were registered from 59 centers across Europe, Russia, and South Africa. In the CA125 guided treatment arm, second-line chemotherapy was started at a median of 4.8 months earlier and third-line chemotherapy with a median of 4.6 months earlier as compared with the treatment arm where therapy was delayed until clinically indicated. Surprisingly, in this study, early treatment on the basis of an early rise in CA125 did not improve survival or quality of life.[44,54] This may reflect the ineffective therapies at the time of the study and illustrates the difficulties in conducting clinical trials over a decade.[54] Results may be invalidated because not all patients received more recent and effective treatments, potentially underestimating the benefit of earlier detection of recurrence.[55,56] In addition, in this trial, CA125 measurements were made in local laboratories rather than centrally, and no information is available about the analytical quality of the measurements and no indication of whether contributing laboratories participated in external quality assessment schemes or compared their results with those of other laboratories. However, all laboratories participated in local quality assurance schemes. According to the requirements of the protocol, all samples from an individual patient were measured in the same laboratory, which is crucial for a trial looking at serial change in marker levels.[55]

The European Group on Tumor Markers (EGTM) has recognized the challenges associated with planning, conducting, and reporting clinical tumor marker surveillance programs and now offers advice on how to design and conduct these types of studies.[57] The European Society of Gynecologic Oncologists has recently advised against universally abandoning CA125 in the routine follow-up of all patients with ovarian cancer. Accordingly, CA125 monitoring should be considered in patients who (i) after complete response on primary treatment have been or are being treated as part of a clinical trial, (ii) would be eligible for (future) clinical trials on second-line treatment, (iii) will not have routine (3 monthly) follow-up including regular imaging, and (iv) are eligible for secondary surgery at recurrence.[44] The current position of the EGTM is that CA125 is recommended for monitoring of patients if surveillance is likely to have clinical consequences (Table 4).

EGTM statement

- CA125 is recommended for monitoring of primary therapy and post-therapy surveillance.
- A CA125 decrement is defined as at least a 50% reduction in CA125 levels from a pre-treatment sample. The decrement must be confirmed and maintained for at least 28 days.
- A CA125 decrement may also be defined by a 50% decrease over four measurements or a 75% decrease over three measurements.
- A CA125 increment among patients with elevated pretreatment concentrations that never normalizes is defined by a doubling of the nadir value.
- A CA125 increment among patients with elevated pretreatment concentrations that normalize is defined by increasing concentrations from below the normal cut-off (35 kU/L) to twice the upper limit of normal (>70 kU/L).
- Alternatively, a CA125 decrement and increment may be based on a statistical estimation of the change adjusted to both analytical and biological variation of CA125.

(LOE III, SOR C)

TABLE 4. Recommendations Published by Different Groups for Use of CA125 in Ovarian Cancer

Use	ACP 1994[17]	EGTM 2005[4]	ESMO 2005[18]	NACB and EGTM 2002[19]
Screening	No	No	None published	NO
Differential diagnosis	None published	Yes (postmenopausal women only)	None published	Yes (postmenopausal women)
Prognosis	None published	No	Yes	Yes
Monitoring therapy	None published	Yes	Yes	Yes
Monitoring follow-up	None published	Yes?	Yes	Yes

ACP, American college of Physicians; ESMO, European Society for Medical Oncology; NACB, National Academy of Clinical Biochemistry; NCNN, National Comprehensive Cancer Network; NIH, National Institutes of Health.

Human Epididymis Protein 4

The HE4 serum levels in healthy women have been reported to range from 60 pmol/L to 150 pmol/L. Reasons for this wide range may be due to the relationship between increasing HE4 serum levels and increasing age.[58,59] Women older than 49 years have higher concentrations as compared with women younger than 40 years.[58] There is a correlation between the histological type and the serum concentration of HE4 with higher concentrations in serous ovarian cancer and with concentrations lowest in patients with mucinous ovarian carcinomas.[58,60] The HE4 in serum has also been identified in pulmonary, endometrial, and breast carcinomas and mesotheliomas, but less frequently in gastrointestinal, renal, and transitional cell carcinomas.[58,61] The most important source of false-positive results in serum is renal failure where concentration of HE4 may be greater than 2000 pmol/l.[62]

HE4 in Differential Diagnosis

Wu et al[63] reported a meta-analysis based on 9 studies evaluating the performance of HE4 among patients with pelvic masses. The pooled sensitivity and specificity of HE4 to diagnose ovarian cancer was 83% (95% confidence interval [95% CI], 77%–88%) and 90% (95% CI, 87%–92%), respectively, when the control group consisted of healthy women. When the control group was composed of women with nonmalignant diseases, the pooled sensitivity and specificity for HE4 was 74% (95% CI, 69%–78%) and 90% (95% CI, 87%–92%), respectively. Li et al[64] reported a review including 2878 patients from 11 studies where HE4 was not superior to CA125 for differential diagnosis. Yu et al,[65] in a meta-analysis including 2607 patients from 12 publications, found that HE4 was better than CA125 for the diagnosis of ovarian cancer in terms of sensitivity and specificity. Hallamaa et al[66] observed no significant variation in serum HE4 concentrations during the menstrual cycle or during hormonal treatment, suggesting that serum HE4 may be measured at any phase of the menstrual cycle and during hormonal treatment with contraceptives. Overall, despite several publications comparing the diagnostic performance of HE4 and CA125 in distinguishing malignant from non-malignant diseases a clear consensus has yet to be reached.[67]

HE4 in Prognosis

Steffensen et al[68] found that an elevated HE4 concentration is a strong and independent indicator of worse prognosis in epithelial ovarian cancer patients as compared with CA125. Elevated serum HE4 levels before therapy significantly correlated with high tumor grade, serous histology, peritoneal involvement, nodal invasion, tumor stage, operative time, and residual tumor size.[69]

The Risk of Ovarian Malignancy Algorithm

In 2009 Moore et al[70] presented the risk of ovarian malignancy algorithm (ROMA), combining HE4 and CA125 in an attempt to predict the risk of serous epithelial ovarian cancer in women with a pelvic mass. They compared the diagnostic

TABLE 5. The Risk of Malignancy Index

Feature	RMI 1 Scoring System[29]	RMI 2 Scoring System[30]
Ultrasound features:	0 = no abnormality	0 = no abnormality
Multilocular cyst	1 = one abnormality	1 = one abnormality
Solid areas	3 = two or more abnormalities	4 = two or more abnormalities
Bilateral lesions		
Ascites		
Intra-abdominal metastases		
Premenopausal	1	1
Postmenopausal	3	4
CA125	kU/L	kU/L

RMI score = ultrasound score x menopausal score × CA125 concentration in kU/L.

RMI > 200 indicates risk of ovarian malignancy. Sensitivities of RMI 1 and RMI 2 were 74% and 80% at specificities of 89% to 92% with positive predictive values of approximately 80%.

NCNN 2007[20]	NIH 1995[21]	NACB 2008[8]	EGTM 2014
None published	No	No (LOE[10] III, SOR B)	No (LOE I), (SOR B)
Yes	Yes (postmenopausal women)	Yes (postmenopausal women) (LOE III/IV, SOR A)	Yes in combination with ultrasound (postmenopausal women) (LOE II-III, SOR B)
None published	Yes	Yes (LOE III, SOR A/B)	Yes (LOE III/IV, SOR B)
Yes	None published	Yes (LOE I/II, SOR A)	Yes (LOE III, SOR C)
Yes	Yes	Yes (LOE III, SOR B)	Yes (LOE III, SOR C)

performance of the ROMA with the performance of the RMI as reported by Jacobs et al and by Bailey et al. The diagnostic performance of the ROMA was similar to the performance of the RMI as reported by Jacobs et al[27] but was superior to the performance of the RMI as reported by Bailey et al[71] Molina et al[58] reported on the sensitivity and specificity of ROMA among 285 patients with nonmalignant gynecological diseases (226 premenopausal and 59 postmenopausal) and 111 patients with ovarian cancer (27 premenopausal and 84 postmenopausal). Among premenopausal women, the sensitivity and specificity of ROMA was 74.1% and 88.9%, respectively. Among postmenopausal women, the sensitivity and specificity was 95.2% and 83.1%, respectively. Van Gorp et al[72] investigated 389 women with a pelvic mass in a prospective study, where 228 women had nonmalignant disease and 161 women had malignant disease. They reported that neither HE4 nor the ROMA performed better than CA125 in the differentiation of ovarian cancer from other pelvic masses. Montagnana et al[73] found preoperative ROMA calculations advantageous when compared to CA125, but found no advantage when compared to HE4. Karlsen et al[74] found that the ROMA and the RMI approach performed similarly in differentiating between nonmalignant and malignant pelvic masses. Further well-designed prospective studies are needed to clarify whether HE4 measurements and the ROMA calculation should be implemented into routine clinical practice.

EGTM statement

• HE4 measurements, either alone or in combinations with CA125, as in ROMA, may be considered for differential diagnosis of pelvic masses especially in premenopausal patients.
(LOE III, SOR B)

In summary

CA125 is not recommended as a routine screening test in asymptomatic women due to a low sensitivity for stage I disease as well as a low specificity especially among premenopausal women.

The RMI 1 and RMI 2 algorithms, particularly in postmenopausal women, are recommended as a way to estimate the probability of malignant potential of a pelvic mass.

The ROMA and the ADNEX algorithms, particularly in premenopausal women, may be considered for estimating the probability of malignant potential of a pelvic mass.

An important application of CA125 is in the monitoring of patients if early recognition of a changing tumor burden has clinical implications.

Reports have indicated an increased specificity of HE4 as a single marker as compared to CA125. The utility in clinical practice should be further clarified.

At present, CA125 remains the best available marker for routine use among patients with serous epithelial ovarian cancer.

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