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SPECIAL ARTICLE

Role of Biomarkers in Screening and Diagnosis of Ovarian Cancer

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ABSTRACT

The overall mortality of ovarian cancer exceeds that of other gynecologic cancers owing to lack of disease-specific symptoms. Three quarters of patients with ovarian cancer are diagnosed after the disease has progressed to advanced stage. Thus, improvement of an effective screening tool could result in better prognosis and survival.

To date, cancer antigen 125 (CA-125) is the most commonly used serum biomarker for detecting ovarian cancer. However, the test has low sensitivity for the disease detection (50-62% for early stage epithelial ovarian cancer) and limited specificity. Therefore, CA-125 as a single test is not recommended for ovarian cancer screening in asymptomatic women.

CA-125 in combination with human epididymis protein 4 (HE4) and Risk of Ovarian Malignancy Algorithm (ROMA) is becoming widely used in clinical practice for the evaluation of adnexal masses. Clinical examination, transvaginal ultrasonography (TVS) and serum biomarkers (ROMA) more effectively classify the patients into low and high risk of ovarian malignancy. The classification allows to direct the patients to appropriate management by gynecologic oncologists, and thus the survival rate could be improved.

Role of serum biomarkers as the screening and diagnosis of ovarian cancer is described in this review.

Keywords: ovarian cancer, screening, CA-125, HE4, ROMA

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Introduction

Ovarian cancer has the highest mortality among gynecologic malignancies owing to lack of disease-specific symptoms in the early stage. Most ovarian cancers are diagnosed at the time when the disease has already been in an advanced stage[1,2]. Diagnosis of ovarian cancer at the earliest stages could enhance the therapeutic efficacy. Therefore, survival rate can be increased through the screening and early detection. Serum tumor markers have a major role in the screening, diagnosis and surveillance of various gynecologic cancers including ovarian cancer. This review focuses on the currently used serum tumor markers' roles in an evaluation of women with adnexal

mass and screening of ovarian cancer among asymptomatic women.

Cancer antigen 125 (CA-125)

CA-125 is a glycoprotein, encoded by the homonymous *MUC16* gene on the chromosome 19. Normal level of serum CA-125 is less than 35 U/mL. Elevated CA-125 may be found in approximately 0.2-5.9% of healthy women[3]. The level tends to decline with aging and menopausal stage. In women with epithelial ovarian cancer, CA-125 is elevated in a greater number of patients with advanced disease stages (stage I: 50-60%, stage II: 80-90% and stages III and IV: more than 90%)[4,5]. In addition, CA-125 is raised in 85% of the serous type and 65% of the endometrioid type. However, only 40%, 36% and 12% of women with clear cell, undifferentiated and mucinous ovarian cancers had elevated CA-125[6]. Apart from ovarian cancers, raised CA-125 level is also found in women during menstruation, pregnancy, and in women with nonmalignant gynecologic diseases including ovarian cyst, endometriosis and uterine leiomyoma. Moreover, elevation of CA-125 has been reported in some nongynecologic diseases, such as inflammatory disorders as well as peritoneal, pleural, musculoskeletal, liver, renal and cardiac diseases[6-8]. Therefore, the specificity of CA-125 as a biomarker for diagnosing ovarian cancer is compromised.

Human epididymis protein 4 (HE4)

HE4 is a glycoprotein, encoded by *WFDC2* gene on the chromosome 20[9]. Normal levels of HE4 vary from 70-150 pmol/L[10,11]. HE4 is expressed in epithelium of normal female genital tissues, including fallopian tubes, endometrium and endocervical glands[12,13]. It is overexpressed in 80-100% of endometrioid, 93-100% of serous and 50-83% of clear cell ovarian cancers, but it is not expressed in germ cell and mucinous ovarian cancers[13,14]. HE4 is also presented in epithelium of respiratory tract, renal convoluted tubules and salivary glands[13,14].

In comparison with CA-125, HE4 is less frequently elevated in benign ovarian tumors and inflammatory lesions. Additionally, HE4 is not changed during the menstrual cycle and its concentration tends to increase with age. Several studies have reported that HE4 is a more specific marker for diagnosing ovarian cancer than other biomarkers including CA-125[10,12,15,16]. HE4 also provides the highest sensitivity among all biomarkers in the diagnosis of ovarian cancer[10]. Furthermore, HE4 in combination with CA-125 has higher sensitivity than other marker combinations for early detection of ovarian cancer[10].

Other biomarkers

Cancer antigen 19-9 (CA19-9) is elevated in 68-83% of mucinous ovarian cancers and in only 28-29% of nonmucinous types[17]. Carcinoembryonic antigen (CEA), cancer antigen 15-3 (CA15-3) and cancer antigen 72-4 (CA72-4) are elevated in 25-50%, 50-56% and 63-71% of patients with ovarian cancer, respectively[17-19].

miRNAs are small non-protein coding gene regulatory RNA molecules. miRNAs in blood circulation of cancer patients are mainly originated from monocytes and exosome released from the tumor[20]. Taylor, et al demonstrated that miRNAs in serum is strongly increased in women with invasive ovarian cancer. In addition, the levels of circulating miRNA-containing exosomes are progressively increased with advanced stages of the disease[21].

Biomarkers for the evaluation of women with adnexal mass

Risk of Malignancy Index (RMI)

RMI was first described by Jacobs, et al in 1990[22]. High RMI in women with adnexal mass indicates high risk of ovarian cancer. Menopausal status, ultrasound characteristics of adnexal mass and serum CA-125 level are used in the formula for calculating the RMI.

RMI = U x M x CA-125

where U indicates ultrasound findings of adnexal mass [1 point for each of the features: solid, multilocular, bilateral tumors, ascites, intra-abdominal metastasis; U = 0 (0 point), U = 1 (1 point) and U = 3 (2-5 points)], M indicates menopausal status [M = 1 (premenopausal) and M = 3 (postmenopausal)] and CA-125 indicates

serum CA-125 concentration in U/mL.

RMI of more than 200 indicates the high risk of ovarian malignancy.

Risk of Ovarian Malignancy Algorithm (ROMA)

ROMA is a test using serum CA-125 and HE4 concentrations and patients' menopausal status to calculate the risk of ovarian cancer[23]. High risk of ovarian malignancy is defined by having ROMA value of more than 13.1% and 27.7% in premenopausal and postmenopausal women, respectively. ROMA is more sensitive than CA-125 or HE4 in the diagnosis of ovarian cancer. ROMA has a sensitivity of 93.8% and specificity of 75% in diagnosing ovarian cancer[24]. Several studies confirmed the effectiveness of this algorithm in the classification of risk of ovarian malignancy[25-27]. The United States Food and Drug Administration (FDA) has approved this algorithm as a useful tool for triaging patients who have an adnexal mass into low and high risk of malignant ovarian tumor[24].

OVA1 test

This test utilizes a combination of 5 biomarkers (CA-125, beta-2 microglobulin, apolipoprotein A 1, transthyretin and transferrin)[28]. It has been approved by FDA since 2009. Scores of higher than 5.0 and 4.4 in premenopausal and postmenopausal women, respectively are associated with high risk of ovarian malignancy.

Biomarkers for screening of ovarian cancers in asymptomatic women

Ovarian cancer screening strategies include transvaginal ultrasonography (TVS) and CA-125 measurement. Currently, there have been 4 major trials in ovarian cancer screening reported.

The Prostates, Lung, Colorectal and Ovarian Cancer (PLCO) trial in the United States of America was a randomized controlled trial of 78,216 women, aged 55-74 years. The enrolled women were assigned to either annual CA-125 screening plus TVS or usual care. This trial showed that CA-125 plus TVS screening did not result in a decrease in the stage of the cancer at the time of its detection and mortality was not different between the 2 groups[29].

The United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) enrolled 202,638 postmenopausal women, aged 50-74 years. The patients were randomly assigned to annual TVS screening (USS group) or annual CA-125 with TVS performed at the time of rising CA-125 level (multimodality or MMS group) or no investigative procedures. This trial demonstrated a decrease in the stage at the cancer detection in women who underwent screening in both USS and MMS groups. In addition, the results showed an overall mortality reduction rate of 20% in the MMS group (a reduction of 8% in years 0-7 and 28% in years 7-14). Moreover, MMS screening was more effective after 7 years of screening[30].

The single arm prospective study, the University of Kentucky Ovarian Cancer Screening Trial included 37,293 asymptomatic women aged 50 years or older and women aged 25 years or older with a documented family history of ovarian cancer. All of them were screened annually with TVS. The trial suggested that annual screening with TVS was associated with decreased stage at the detection of cancer and reduction in ovarian cancer mortality[31].

The Japanese study was a prospective randomized trial in which asymptomatic postmenopausal women were allocated either to a screening group using annual TVS and CA-125 level or a control group. The proportion of stage I-II ovarian cancers was higher in the screening group (67%) than in the control group (44%), but this difference did not reach statistical significance ($p = 0.116$)[32].

New histopathological, molecular and genetic studies have recently provided 2 categories of ovarian carcinogenesis[33]. Type I carcinomas are often early stage and low-grade tumors, with a relatively indolent disease course. These tumors often develop from benign ovarian cysts. Type II carcinomas are high grade and more clinically aggressive. *BRCA* genetic mutations and other germline mutations are associated with type 2 carcinomas. The conclusion from these major ovarian cancer screening trials suggests that annual screening by using CA-125 and TVS may be

useful for detecting a precursor of type I ovarian cancer. Annual screening may result in a decrease in the stage at the cancer detection and an improvement in prognosis of type II carcinomas. A combination of CA-125 and TVS is not cost-effective, thus it is still not routinely used for the screening of ovarian cancer in asymptomatic population. However, CA-125 in combination with TVS may have a role in an early detection of ovarian cancer in women with *BRCA-1* and *BRCA-2*-related hereditary ovarian cancers.

Conclusion

Early stage of ovarian cancer is often asymptomatic. The challenge in developing biomarkers or other effective screening tools for early diagnosis of ovarian cancer still exists. CA-125 combined with HE4 and ROMA are becoming widely used in clinical practice for the evaluation of adnexal masses. Clinical examination, TVS and ROMA more accurately classify the patients into low and high risk of ovarian malignancy. As a result, improved outcome of ovarian cancer management could be achieved.

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