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REVIEW PAPER

Current clinical application of serum biomarkers to detect ovarian cancer

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Abstract

For the last decades, hundreds of potential serum biomarkers have been assessed in diagnosing of ovarian cancer including the wide spectrum of cytokines, growth factors, adhesion molecules, proteases, hormones, coagulation factors, acute phase reactants, and apoptosis factors but except CA125 none of them have been applied to everyday clinical practice. Nowadays, the growing number of evidence suggests that the classic marker CA125 should be accompanied by HE4 and in fact, Risk of Ovarian Malignancy Algorithm (ROMA) is becoming more and more widespread in clinical practice for the evaluation of adnexal masses. Early ovarian cancer is often asymptomatic, so the challenge still exists to develop serum markers suitable for early diagnosis and screening. Current knowledge strongly points to different mechanisms of pathogenesis, genetic disturbances and clinical course of major histological subtypes of ovarian cancer. Thus, future biomarker/multimarker panels should take into consideration the implications of different molecular patterns and biological behavior of various subtypes of ovarian cancer. Very promising are studies on miRNAs – small non-protein coding gene-regulatory RNA molecules functionally involved in the pathogenesis of cancers acting as oncogenes (oncomirs) or tumor suppressors. The studies devoted to ovarian cancer tissue miRNA profiling have shown that miRNAs could be useful in diagnosing and predicting the OC outcome. They also confirmed that OC is a highly heterogeneous disease, gathering four distinct histological tumor subtypes characterized not only by distinct origin, behavior and response to chemotherapy but also by different patterns of miRNA expression.

Key words: ovarian cancer, tumor markers, CA125, HE4, ROMA, OVA1, miRNAs.

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]. Population risk of ovarian cancer is about 1-1.5%, what means that the great majority of adnexal tumors is benign and can be operated by general gynecologists [3]. However, many benefits have been proven for patients with ovarian cancer operated by oncological gynecologists compared with general gynecologists and surgeons (more accurate staging, more precise cytoreductive surgery, smaller number of complications, higher percentage of 5-year survival) [4-6]. Therefore, women with suspected ovarian tumors should be directed to centers specializing in oncological gynecology.

On that ground, new methods of selecting patients at a high risk of ovarian malignancy are investigated. Nowadays, physical bimanual examination, gynecological 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 are more objective and comparable.

In the past years, a wide spectrum of cytokines, growth factors, adhesion molecules, proteases, hormones, coagulation factors, acute phase reactants, apoptosis factors were investigated as potential single serum biomarkers and in multimarker panels in diagnosing ovarian cancer, but except CA125, none of them have been applied to everyday clinical practice.

CA125

CA125 (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 only 12% of mucinous ovarian cancers [8]. For the last three decades it has become the most widespread biomarker of ovarian cancer. The utility of CA125 in the diagnosis of ovarian cancer has been evaluated in a large number of studies.

Serum levels of CA125 are within the normal limits in at least 20% of patients with ovarian cancer and about

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a half of patients in its early stages, which considerably 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]. CA125 levels are also raised in patients with other gynecological diseases (such as myomas of the uterus, benign and borderline ovarian tumors), many non-gynecological 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, a large number of researches have been conducted to improve the ovarian cancer diagnostic protocol.

Attempts were 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, first of which (RMI I) occurred to be the most efficient [14-17]. It is calculated with the use of following formula:

$$RMI = U \times M \times CA125,$$

where: *U* – ultrasound image (1 point for each of the features: solid, multilocular, bilateral tumor, 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]. RMI has become a practical diagnostic tool, which is still used in the diagnosing of ovarian tumors.

HE4

HE4 (human epididymis protein 4) is a glycoprotein encoded by WFDC2 gene (chromosome 20) [18]. Presumably, it takes part in immune response, but its role has not been precisely specified yet [19, 20]. It is present in the epithelium of fallopian tubes, endometrium, endocervical glands, but not in ovarian surface epithelium. Expression of HE4 has also been noted in epithelium of the respiratory tract (especially 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 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 tumors, 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]. Serum concentrations of HE4 are also more rarely increased than CA125 in serous cysts, teratomas, fibromas, inflammatory lesions. However, serum levels of both CA125 and HE4 do not show an statistically relevant difference in patients with mucinous cysts [9]. Because HE4 levels are not affected by many benign diseases which increase concentrations of CA125, the new marker may be a valuable complement in distinguishing malignant from benign ovarian tumors.

In contrast to CA125, HE4 concentrations are lower in pregnant women when compared with their premenopausal counterparts. Levels of HE4 do not differ significantly between trimesters. Only between second and third trimester of pregnancy, a slight statistically insignificant increase in the HE4 concentrations was reported [23].

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 defined well yet [24].

HE4 levels do not alter during the menstrual cycle, so they can be determined regardless of its phase [24]. Among healthy women, concentrations of HE4 increase with age (starting from the age of 40), especially in the 8th and 9th decade of life. This fact ought to be taken into account, when serum of elderly women is tested, because elevated levels of HE4 may lead to false-positive results in this group of patients [23].

Unfortunately, the normal limits for HE4 have not been established well yet. Depending upon the study, normal ranges vary from 70 to 150 pM [1, 25]. Moore *et al*. have determined normal values separately for patients before and after menopause (89.1 pM and 128.0 pM, respectively) [23].

ROMA

Based on the encouraging results of HE4 in the diagnosis of ovarian cancer, especially in combination with CA125, Moore *et al*. have 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 tumor. Cut-off values differ slightly depending on the manufacturer of the diagnostic kit.

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 diagnosis of ovarian cancer (94.3% vs. 84.6% at 75% specificity). When early stages of ovarian cancer (FIGO I/II) were concerned, the difference between these two tests was even more remarkable (85.3% for ROMA vs. 64.7% for RMI at 75% specificity) [26].

Many studies evaluating 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 [27, 28]. Most of available meta-analyses show similar findings [29-31]. 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 three methods (specificity of 87% for HE4 vs. 82% for ROMA vs. 76% for CA125) [31]. However, due to heterogeneity of studies, it is stressed that the results of meta-analyses should be treated with caution [29-31].

OVA1

In 2009, the U.S. Food and Drug Administration approved for clinical use a new test, OVA1 [32]. It evaluates serum concentrations of five markers. Two of them are upregulated (CA-125 II, β-microglobulin) and three 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. The value ≥ 5.0 in premenopausal and ≥ 4.4 in postmenopausal women qualify patients to the high risk group. Patients with a positive result of OVA1 test should be referred to an oncological gynecologist [33].

Results of a multicenter study (OVA500 Study) showed 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 OVA1 test is noteworthy [34].

A little number of studies evaluating utility of the OVA1 test have been published. However, available publications confirm to some extent 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 [35]. In one study, OVA1 qualified to the high risk group 76% of patients with malignant adnexal tumors who had serum CA125 levels within the normal limits [36]. Some reports also confirm high sensitivity of the OVA1 test in the diagnosis of early ovarian cancer [37]. Up till now, there are hardly any available studies directly comparing performance of the OVA1 test and ROMA.

Future perspectives: circulating miRNAs

The last decade has brought better understanding of heterogeneity of ovarian cancer. Future, improved biomarker and multimarker panels should be based on molecular origin of various OC subtypes. Very promising are studies on miRNAs – small non-protein coding gene-regulatory RNA molecules functionally involved in the pathogenesis of cancers acting as oncogenes (oncomirs) or tumor suppressors.

The studies devoted to ovarian cancer tissue miRNA profiling have shown that miRNAs could be useful in diagnosing and predicting the OC outcome [38, 39]. They also confirmed that OC is a highly heterogeneous disease, gathering four distinct histological tumor subtypes characterized not only by distinct origin, behavior and response to chemotherapy but also by different patterns of miRNA expression [40]. From the clinical and practical point of view a more interesting and important question is whether the miRNAs could be used as attainable biomarkers (alone or combined with other markers i.e. CA125) present in peripheral blood.

MiRNA can pass between tissues and organs through blood circulation. The source of miRNAs found in the peripheral blood of cancer patients is tumor tissue. Circulatory miRNAs originate mainly from monocytes and exosomes/microvesicles released from the tumor [41, 42]. miRNAs are incorporated into a membrane-enclosed complex or bound to proteins, therefore, are resistant to plasma RNases and stable against temperature and pH changes [43, 44]. These features make them reliable candidates for diagnostic and predictive biomarkers. In most opinions circulating miRNAs are malignancy-type specific and are characterized by the same miRNA signature as the parental tumor [45]. Therefore, the majority of circulating miRNA biomarker

studies are based on the primary tumor miRNA expression profiles. However, the lack of correlation between paired tissue-plasma miRNA expression profiles in some studies strongly suggests that malignant tumor cells are not the sole source of circulating miRNAs. It is possible that circulating miRNAs signatures are affected and changed by loco-regional inflammation observed in many tumors, and that the final result of miRNA profiling in blood is a mixture of tumor-specific and inflammation-specific miRNAs [46].

The first attempts to use serum miRNA as cancer biomarkers were described in patients with diffuse large B-cell lymphoma and indicated that levels of miR-21 correlated with patients' relapse-free survival [47]. Subsequently, serum miRNAs were tested as biomarkers for monitoring in prostate cancer (miR-141) [41], followed by studies of miRNA use for the early detection both in the lung and colorectal cancer based on miR-25 and miR-223 serum concentrations [43, 48]. Nowadays, similar studies have been performed in many types of cancer including breast cancer, gastric cancer and ovarian cancer (OC) [48, 49].

The first study in OC was performed by Taylor *et al*. [50] who found that miRNAs over-expressed in serous ovarian cancer tissue (miR-21, miR-141, miR-200a, miR-200c, miR-200b, miR-203, miR-205, and miR-214) were similarly elevated in serum-derived exosomes. The results differed significantly when compared to the control group of patients with benign ovarian pathology [50]. In another study, Resnick *et al*. [14] tested serum miRNA expression of twenty one miRNAs selected from the epithelial ovarian cancer profile. They found that five miRNAs were overexpressed (miR-21, miR-29a, miR-92, miR-93, and miR-126) and three were underexpressed (miR-127, miR-155, and miR-99b) in the pre-surgical serum of ovarian cancer patients when compared to normal controls. What is interesting, three of miRNAs, namely miR-21, miR-92 and miR-93, which probably functioned as the oncomirs, were significantly overexpressed in patients with normal pre-operative CA125 levels. This suggests that some miRNAs could be complementary biomarkers in patients with negative results of routine tests [39, 45, 48]. Overexpression of members of miR-200 family (miR-200a, miR-200b, and miR-200c) was confirmed in serum of serous OC patients by Kan *et al*. [51]. The predictive model constructed on the basis of miR-200 expression, in the opinion of authors, was able to discriminate patients with high-grade serous OC from age-matched healthy controls [51]. According to some investigators, the use of the miR-200 family is a promising direction in diagnosis and monitoring of therapy in OC patients [45]. The research performed by Chung *et al*. [52] was planned to examine in the microarray analysis and qRT-PCR a total RNA isolated from serum, tissue and ascites in serous OC patients. Expression of four serum miRNAs (miR-132, miR-26a, let-7b, and miR-145) was found to be significantly down-regulated in OC patients compared to controls, making these miRNAs potential candidates for novel biomarkers in serous OC [52]. An interesting study conducted by Suryawanshi *et al*. [46] compared the plasma miRNA profiles in endometriosis, endometrioid and serous ovarian cancer. The results of this study confirmed that endometrioid and serous OC are distinct entities and they can be distinguished based on plasma miRNA profile. Moreover, a trend of miRNA changes from endometriosis to endometrioid OC was shown [46]. A combination of miR-21, miR-362-5p, and miR-1274a enabled differentiation between endometriosis and endometrioid OC (57% sensitivity, 91% specificity), while miR-21, miR-191, and miR-1975 together could distinguish between endometrioid and serous OC (86% sensitivity, 79% specificity) [46]. The other combinations of miRNAs could further differentiate the studied population, as follows: miR-16, miR-191 and miR-195 – healthy vs. endometriosis; miR-16, miR-21, and miR-191 – healthy vs. endometrioid OC; miR-16, miR-191, and miR-4284 – healthy vs. serous OC; miR-362-5p, miR-628-3p, and miR-1915 – endometriosis vs. serous OC [46]. Recently Ji *et al*. [53] identified eleven up-regulated and nineteen down-regulated miRNAs in sera of OC patients. Among them miR-22, miR-93, mir-106b and miR-451 were validated quantitatively. It was found that miR-22 and miR-93 were consistently up-regulated more than 2-fold in OC cases. Expression of miR-106b was significantly lower, whereas that of miR-451 was higher in the group of patients over 51 years of age, what was a novel and interesting discovery. Moreover, authors showed that miR-106b and miR-93 serum concentrations were down-regulated in patients with the highest CA125 levels, although there was no connection with the clinical stage of the disease [53]. Another recent study by Shapira *et al*. [54] for the first time focused on protein-bound miRNA in plasma free of cellular debris, microvesicles or exosomes. Analysis showed that nineteen miRNAs were down-regulated while three were up-regulated in serous OC compared to control healthy subjects. Among them six miRNAs (miR-106b, miR-126, miR-150, miR-17, miR-20a, and miR-92a) were significantly decreased in OC patients [54]. The results of all cited above studies are summarized in Table I (adopted from [45] and updated).

Conclusions

For the last decades hundreds of potential serum biomarkers have been assessed in diagnosing of ovarian cancer. Nowadays, the growing number of evidence suggests that the classic marker CA125 should be accompanied by HE4 and in fact, ROMA algorithm is becoming more and more widespread in clinical practice for the evaluation of adnexal masses. The second mul-

Tab. I. Studies on circulating miRNAs as potential biomarkers of ovarian cancer

Author/Reference no.	Year	Ovarian cancer type	Control	Type of sample	Up-regulated	Down-regulated
Taylor and Gercel-Taylor [50]	2008	Serous	Benign ovarian tumors	Exosomal serum miRNA	miR-21, miR-141, miR-200a, miR-200b, mir-200c, miR-203, miR-205, miR-214	
Resnick *et al.* [48]	2008	Generally epithelial ovarian cancer	Healthy controls	Serum miRNA	miR-21, miR-92, miR-93, miR-126, miR-29a	miR-155, miR-127, miR-99b
Kan *et al.* [51]	2012	Serous (high grade)	Healthy controls	Serum miRNA	miR-200a, miR-200b, miR-200c	
Chung *et al.* [52]	2013	Serous	Healthy controls	Serum miRNA		miR-132, miR-26a, Let-7b, mir-145
Suryawanshi *et al.* [46]	2013	Endometrioid/Serous	Healthy controls/Endometriosis	Plasma miRNA	miR-16, miR-21, miR-191, miR-4284	
Ji *et al.* [53]	2014	Generally epithelial ovarian cancer	Healthy controls/Benign ovarian tumors	Serum miRNA	miR-22, miR-93	
Shapira *et al.* [54]	2014	Serous	Healthy controls/Benign ovarian tumors	Plasma miRNA		miR-106b, miR-126, miR-150, miR-17, miR-20a, and miR-92a

timarker panel approved by FDA – OVA1 is not widely used outside the USA. Taken together, clinical examination, ultrasonography and serum markers (ROMA) more accurately select the patients with a high risk of ovarian malignancy what allows to direct them to centers specializing in oncological gynecology.

Such diagnostic workup is possible when the woman comes to the gynecologist and the adnexal mass is diagnosed. Ovarian cancer at the beginning gives unspecific mild signs or no symptoms at all. In fact early ovarian cancer often is asymptomatic, the challenge still exists to develop serum markers suitable for early diagnosis and screening.

Current knowledge strongly points to different mechanisms of pathogenesis, genetic disturbances and clinical course of major histological subtypes of ovarian cancer. Thus, future biomarker/multimarker panels should take into consideration the implications of different molecular patterns and biological behavior of major subtypes of OC.

Disclosure

Authors report no conflict of interest.

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