RESEARCH ARTICLE

Ovarian Cancer - Current Status of Blood Biomarker and Imaging Screening Strategies

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Abstract
Ovarian cancer is most lethal of all the gynecologic malignancies and the fifth leading cause of cancer deaths in women overall, accounting for about 5% of female cancer deaths. To improve the outcomes, an efficient screening tool for early detection of the disease at an earlier, curable stage would be required. Since the vast majority of ovarian cancer cases are sporadic in nature with relatively low incidence, a screening test has to offer very high specificity to avoid unnecessary interventions in false-positive cases to be considered suitable for general population use. Another approach to screening would entail increasing the pretest likelihood by focusing on patients at increased risk only. Several larger scale, randomized controlled trials are working on establishing screening strategies for the general population with the most promising results so far shown by the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS), which revealed improvement in survival rates and reduction in mortality within screened patients. However, more data is required to establish the benefits, warranting further validation. Currently, research is ongoing on OC screening benefits and developing a suitable algorithm in order to have better patient outcomes. In this review article, we discuss current updates on OC screening strategies with special focus on novel development in biomarkers and sonography, as screening tools.
Keywords: Cancer Antigen-125, Contrast Enhanced Ultrasound, Ovarian Cancer, Serum Human epididymis 4, Transvaginal Ultrasound, Ultrasound Molecular Imaging

Abbreviations
ACC: Accuracy
CA-125: Cancer Antigen-125
CEUS: Contrast Enhanced Ultrasound
HE4: Human epididymis 4
NPV: Negative predictive value
OC: Ovarian Cancer
PLCO: Prostate, lung, Colorectal and Ovarian randomized trial
PPV: Positive predictive value
RCT: Randomized Control Trial
ROCA: Risk of Ovarian Cancer Calculation Assessment
ROMA: Risk of Ovarian Malignancy Algorithm
TVUS: Transvaginal Ultrasound
UKCTOCS: United Kingdom Collaborative Trial of Ovarian Cancer Screening
USMI: Ultrasound Molecular Imagin

1. Introduction

In 2020, there will be approximately 21,750 new diagnosed cases of ovarian cancer and 13,940 ovarian cancer (OC) deaths in the United states, accounting about 5% of female cancer deaths estimated by the American Cancer Society. It is the fifth most common cause of death in women due to its low survival rates resultant of late detection of most cases.[1,2] Despite improved and ever more aggressive therapy approaches, overall ovarian cancer 5 year survival is about 40%, but it could be up to 93% when the disease is diagnosed at an early stage (confined to the ovary); however, at present we are able to diagnose only 20-25% of OC cases at an early stage.[3,4] Since the vast majority of ovarian cancer cases are sporadic in nature with relatively low incidence, apart from low cost a screening test has to offer very high specificity in addition to high sensitivity to avoid unnecessary interventions in false-positive cases in order to be considered suitable for use in the general population. Another approach to screening would entail increasing the pretest likelihood by focusing on patients at increased risk only.[5,6] Currently, there is no established screening test for OC detection for either screening scenario; however, some large scale randomized controlled trials (RCTs) evaluated potential screening tools for use in the general female population. The aim of these trials is to evaluate whether variable screening approaches detect ovarian cancer at an earlier and curable stage in order to increase long-term survival and reduce mortality rates.[7-10] In this review article, the authors discuss current status of OC screening strategies with special focus on biomarkers and sonography, as screening tools.

2. Ovarian cancer screening Trials

The United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) and the Prostate, Lung, Colorectal and Ovarian randomized trial (PLCO) are the two major randomized controlled screening trials that have reported the efficiency of these trials in early diagnosis of OC and mortality benefits, if any.[7-12] In both trials annual screening was performed in asymptomatic postmenopausal women aged 55-74 years selected from general population and screened by assessing CA-125 level and/or performing TVUS; however, both have conflicting preliminary results and require further follow up. Table 1 illustrates the comparison of both studies.

The UKCTOCS is the largest screening trial (allocated a total of 202,638 women), which shows promising results of OC screening. This trial started enrollment in 2001 and divided screening population into two groups, the multimodality screening group (MMS) and TVUS group (USS). The participants were included into MMS, USS and control group at a ratio of 1:1:2 in a randomized fashion. In the MMS arm, each participant first underwent assessment of serum CA-125 levels applying the Risk of Ovarian Cancer Calculation Assessment (ROCA) algorithm (figure 1), which interprets interval changes in serum CA-125 concentration above every individual's baseline value and classifies results into three risk categories: Low, intermediate and elevated risk. Participants with elevated CA-125 were followed by second level screen by TVUS. The USS arm had only one TVUS test. The two screening arms were compared with a no screening control group. All these participants were followed for a median 11.1 years and results favoured the MMS arm over the USS arm for detection of both primary OC/FT cancer and primary invasive OC/FT cancer with specificities of 99.8% and sensitivities of 89.4 and 89.5%, respectively, at 95% confidence interval (CI).[7] Although, the mortality reduction was not significant in

the initial screen, it was significant when the prevalent cases were excluded. An overall mortality reduction of 20% and a reduction of 8% in years 0-7 and 28% in years 7-14, favouring the MMS study arm.[7,11,12]

The PLCO trial (which enrolled a total of 78,216 women) had a single screen arm in which participants were evaluated with CA-125 and TVU and compared with no screening/usual care control group. A single cut-off value of 35 U/ml CA-125 was considered as positive screen and the median follow up was 14.7 years for OC diagnosis and mortality. PLCO results were not favourable, only 28% of the OC cases were diagnosed in early stages (Stage I/II) and showed no true mortality benefit. Even when retroactively applying the ROCA to the screening arm of the PLCO trial, there was no statistically significant mortality benefit of ovarian cancer screening.[10] Moreover, 3285 (5%) screened patients showed false positive results, of which 1080 underwent surgery and 163 (15%) had at least one serious complication.[8-10,12]

The Japanese Shizuoka Cohort study for OC screening is an another RCT study (enrolled a total of 82,487 women) which also used a single screening arm, similar to that of PLCO, however added physical exam to the usual tools, CA-125 and TVU. They also used a single cut-off value of 35 U/ml for analysing CA-125 levels and followed up their participants for about 9.2 years. Interestingly, the result was promising for early detection of OC, unlike the PLCO trial, 63% of OC were detected at stage I in the screening arm versus 38% in the control arm. But no overall mortality benefit was documented.[13]

3. Ovarian cancer screening tools: Current status and future prospects

Despite extensive research of potential blood biomarkers for ovarian cancer, **Cancer antigen-125 (CA-125)** remains the single best marker so far followed by **Human Epididymis Protein 4 (HE4**) with reported sensitivities of 86% and 73% respectively at 95% specificity.[14,15] Both markers are FDA approved for use in the Risk of Ovarian Malignancy Algorithm (ROMA), which combines use of both HE4 and CA-125. ROMA had higher sensitivity (94.1% at 90% specificity) for more aggressive invasive epithelial type II OCs, similar to the model which combined TVUS, CA-125 and HE4. This supports the use of HE4 as an alternative to TVUS where it may not be available readily or to reduce the number of tests.[15-17]

3.1 Cancer antigen-125 (CA-125) is a high molecular weight glycoprotein member of the mucin family that is seen in the bloodstream in cases of epithelial ovarian cancer. It can be elevated in about 50% of early OC (stage I) and up to 90% in advanced OC.[18] There are multiple theories related to CA-125 cut off value and its significance regarding tumour size. A cut off value of 35 U/ml is considered significant and tumour size of 3 mm is considered sufficient to generate a positive biomarker screen as shown in several mathematical biomarker models.[18-20] However, the UK collaborative trial of ovarian cancer screening (UKCTOCS) uses the ROCA approach which is based on every woman having her own individual baseline value and incorporates serial change in biomarker levels over time into the cancer screening strategy. CA-125 blood level changes equal or greater than 30 U/ml above baseline were identified as significant.[7,11,21] Originally approved for surveillance and recurrence detection in treated ovarian cancer patients, it has not been approved so far as a standalone

screening test because of low sensitivity and specificity, since it can be found in 1% of normal population, 6% of benign diseases and 28% of non-gynecological malignancies. Thus, it still remains under research for use as a screening tool.[4,18]

3.2 Serum Human epididymis 4 (HE4), a secreted glycoprotein (belongs to a family of proteins which typically function as proteinase inhibitors), which is overexpressed by epithelial ovarian cancers. It has gained attention recently and has been approved by the US Food and Drug Administration for its use in diagnosing and monitoring treatment response in women with OC. Multiple studies have shown that the serum HE4 has similar diagnostic sensitivity and a higher specificity to that of CA-125.[16,22,23] Lin et al reported a specificity of 87% for HE4 in comparison to CA-125 with a specificity of 76% .[24] The role of HE4 in diagnosis, prognosis and follow-up of OC was discussed in a systematic review by Scaletta et al.[16] They reported a higher sensitivity (64%) in early diagnosis of epithelial OC as compared to CA-125 (45.9%). It was approved in combination with CA-125 as part of the risk of the Risk of Ovarian Malignancy Algorithm (ROMA™) test for determining ovarian cancer risk in pre- and post-menopausal women. When this approach was used as a means to differentiate between benign and malignant masses in 322 patients with benign and 327 patients with invasive ovarian tumors, a benefit over CA-125 alone in differentiating benign and invasive entities was shown in premenopausal, but not in postmenopausal women.[25] Similarly, a recent cohort study suggested limited role of incorporating HE4 to concurrent use of CA-125 and TVU in differential diagnosis of adnexal masses in post-menopausal women.[17]

More successful than individual blood biomarkers may be an entire panel of biomarkers such as examined in a subgroup of 49 ovarian cancer and 31 control patients of the UKCTOCS.[26] In that study, a combination CA125, HE4, CHI3L1, PEBP4 and/or AGR2, resulted in 85.7% sensitivity at 95.4% specificity up to 1 year before diagnosis of ovarian cancer, which was a considerable improvement over the use of CA-125 alone (64.3 % sensitivity at 95.4% specificity). Another panel that was shown to add some value in combination with CA-125 was HE4 and CA72-4).[27] At 98% specificity, these three markers in combination detected additional 4 out of 25 screen negative cases compared to CA-125 alone.

Overall, more studies with higher sample size are needed to prove the significance of using HE4 alone or in conjunction with CA-125 and TVU as well as the suitability of more novel biomarker panels. None of the biomarkers has been established as a standalone screening tool in general or high-risk patients. Integrating specific biomarkers with an imaging technique in the context of multimodality screening may likely be more appropriate for screening patients and most of the major ovarian screening trials are based on this strategy.[7-9,13,28]

4. Ultrasound Imaging

4.1 Transvaginal Ultrasound (TVUS) is a relatively cost-effective and widely available screening tool and when used as a second step in conjunction with the biomarker CA-125 can to some degree reduce false positive results, in cases when CA-125 is positive in benign conditions like endometriosis, adenomyosis, pelvic inflammatory disease and leiomyoma. This makes TVUS a favorable tool in a multimodality screening approach to confirm the presence and localization of disease in case of a positive CA-125 screen. As a real-

time imaging test, TVUS is already the established first line tool for ovarian imaging that can depict volume and morphologic changes in high resolution. Morphology changes which favor malignancy include complex cystic lesions with solid components, thick walls and/or septations >3mm, papillary projections or solid ovarian tumors with increased organ volume. However, the specificity of TVUS remains somewhat low as there is considerable overlap between imaging findings in benign and malignant masses. Extensive research has gone into scoring of the imaging findings for risk stratification purposes. The specificity can be improved by various measures: 1. Repeating the TVU exam after six to eight weeks of the first exam to exclude physiologic, reversible changes, 2. characterizing lesions according to one of the established risk stratification scoring systems, such as the International Ovarian Tumor Analysis (IOTA) or recently introduced Ovarian-adnexal Reporting and Data system (O-RADS).[29-31] Both of these scoring systems use Doppler and conventional B-mode ultrasound.

In 2008, the IOTA group described the Simple Rules classification system, based on a set of 5 ultrasound B-features (unilocular cysts, solid components <7mm, acoustic shadows, smooth multilocular tumors <100mm, no intrinsic blood flow; color score 1) and of 5 M-features (irregular solid tumors, presence of ascites, at least 4 papillary structures, irregular multilocular-solid tumors =/>100mm, very strong intrinsic blood flow; color score 4) and reported that these rules can correctly classify adnexal masses into benign and malignant tumors, respectively, in a large percentage of cases.[28] That group further predicted the risk of malignancy in adnexal masses implementing the Simple Rules from IOTA in an international multicenter study on the imaging data of 4848 patients and calculated that 23% of patients had a low estimated risk (<1%) and 48% had a high estimated risk (=/>30%) of malignancy. This formed the basis for choosing the optimal treatment, with the high risk patients to be referred to gynecological oncologist for surgery, while low risk patients managed locally with a conservative approach.[32] This approach further evolved into the mathematical predictive ADNEX model for presurgical risk stratification of ovarian tumors.[33] The ADNEX model aims at classifying the tumors in greater detail into benign, borderline, and various stages of malignant tumors. But adoption of the mathematical model was relatively low in the United States and the desire for a morphologic, pattern recognition risk assessment scoring system then, ultimately, lead to the creation of the Ovarian-Adnexal Reporting and Data System (O-RADS) lexicon by a consensus panel of experts using IOTA descriptors. O-RADS offers optimized standardized terminology in gynecological imaging reporting and a US risk stratification and management system.[30,31] In O-RADS, each lesion type is assigned a score (O-RADS 0 to 5) based on the US descriptors and risk assessment, which further guides the appropriate management for each lesion. The patients with suspicious lesions are to be referred to gynecologic-oncologist for surgery versus more conservative management for benign-appearing lesions, hoping that these measures can reduce the number of unnecessary interventions.[4,34,35]

4.2 Contrast enhanced Ultrasound (CEUS) with the use of nontargeted microbubbles have shown promising results in determining malignancy in conventional US indeterminate adnexal masses by adding physiologic information on tumor vascularity. Most commonly used microbubbles have a core of gas (e.g. perfluorocarbon, nitrogen) encapsulated by

tight biopolymer or lipid-galactose stabilized shells and are generally very safe in clinical use.[18] These microbubbles have the ability to oscillate, change their size and shape, thus generating a strong acoustic signal when exposed to the acoustic field. A single center study on 120 patients was performed and found early or simultaneous inhomogeneous enhancement resulted in differentiating malignant from malignant masses with the sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV) and accuracy (ACC) of 89.6%, 97.2%, 93.2%, 95.6% and 93.3%.[36] Another study established 3D-CEUS scoring system using 51 small adnexal masses (<4cm) and concluded that a specific cut-off score of =/> 8 suggested malignancy. This scoring system had a sensitivity and specificity of 100% and 98%. However, both the above studies used qualitative methods to assess the malignancy in the adnexal masses, which can be affected by interobserver variability.[37] To further prove the efficacy of CEUS in differentiating adnexal masses, Szymanski et al studied quantitatively the relationship between contrast kinetics in tumor vessels and lesion histologic type using 50 adnexal masses and reported significantly higher baseline, maximum color Doppler, absolute and relative increase in color Doppler intensities in malignant lesions versus benign lesions with an estimated PPV of 97.1%, NPV of 100% and ACC of 100%.[38] For further validation of utility of CEUS in evaluating adnexal masses, large scale multicentric studies are required.[39]

A comprehensive meta-analysis study was performed to assess the diagnostic value of TVUS, Doppler and contrast enhanced Ultrasound (CEUS) in differentiating benign from malignant ovarian masses in 100 studies evaluating a total 8,819 patients and the study showed a pooled sensitivity of 92% for TVUS, 93% for Doppler US and 97% for CEUS, a pooled specificity of 86% for TVUS, 85% for Doppler US and 92% for CEUS and Area under the curve (AUC) as 95% for TVUS, 96% for Doppler US and 99% for CEUS.[34] That said, CEUS has huge potential in evaluating the different ovarian tumor entities; however, the major limitation of these imaging modalities is their inability to diagnose OC in its earliest stages, especially the more aggressive invasive epithelial carcinomas and/or when the size of tumor is still very small when it does not yet stand out of the regular heterogenous ovarian architecture on conventional B-mode ultrasound.[4,34,35] This leaves a gap of opportunity for further improvement and development in ultrasound imaging.

4.3 **Other cross-sectional imaging modalities** such as computed tomography (CT), magnetic resonance imaging (MRI) and positron emission tomography/computed tomography (PET/CT) offer the advantage of operator independence over ultrasound. MRI also offers excellent soft tissue contrast. But these imaging modalities are either cost-prohibitive in the case of MRI and PET/CT or expose the patient to ionizing radiation in the case of CT and PET/CT which renders these modalities unsuitable for the use in a screening setting where repeated imaging of asymptomatic patients is required[18].

4.4 Ultrasound molecular imaging (USMI) is a novel technology that allows quantification of target molecules that are over-expressed on tumor-associated microvasculature endothelial cells.[14,18,40-42] This imaging method exploits the fact that for growth beyond a size of typically 1-2 mm, solid tumors require the formation of multiple new blood vessels, since at that size diffusion is no longer sufficient for further tumor growth. Hence, tumor cells produce multiple molecules that enhance vascular proliferation and in return tumor-associated vasculature

over-expresses receptors for these pro-angiogenic factors in the context of tumor angiogenesis. In molecular ultrasound**,** the surface of contrast microbubbles is functionalized with high affinity ligands that are targeted to specific surface antigens over-expressed on the tumor-associated vascular endothelial cells. Among the many possible pro-angiogenic molecules in the tumor microvascular environment, Vascular Endothelial Growth Factor is considered one the most important and thus Vascular Endothelial Growth Factor Receptor Type 2 receptors (VEGFR2/KDR) are usually highly overexpressed in solid tumors, although this phenomenon is not entirely specific for tumor angiogenesis.[18,43-45] In animal models, strong signal from VEGFR2-targeted binding microbubbles can be seen in tumors as small as 2-3 mm in diameter.[40,46]

Recently, a first-in-human clinical study was performed to evaluate the expression levels of KDR via USMI and immunohistochemistry in patients with breast and ovarian cancers.[42] Strong KDR-targeted USMI signal was observed in 77% of malignant ovarian cancers and no or low KDR-targeted signal in 78% of benign ovarian lesions. Additionally, the KDR expression on IHC matched the USMI signal in 85% of malignant ovarian lesion. After the promising results of this study, a new trial of VEGFR2-targeted contrast-enhanced ultrasound is currently underway [47]. The aim of this trial is to explore the performance of VEGFR2-targeted USMI in patients with suspected ovarian cancer/complex adnexal lesions and also in patients with healthy ovaries and results are expected in 2-3 years.

5. Conclusion

A multimodality screening strategy that combines change over time of a blood biomarker or likely panel of biomarkers with a suitable low cost, widely available imaging method holds the greatest potential for improving ovarian cancer outcomes. While the cost and performance of such a strategy currently still seem problematic when used for screening in the general population, it may be more successful in defined patient populations at increased risk for the disease. So far, large scale studies using multimodality screening approaches combining single blood biomarkers followed by TVUS have shown mild increase in detection of ovarian cancer at earlier stages, but not yet shown a clear decrease in mortality from the disease. Adding information on the molecular footprint of the ovarian tissue to TVUS by using molecularly targeted CEUS is a newer development that is currently under investigation. It has the potential for higher sensitivity and specificity when added to conventional TVUS, but this method is still in an early stage and further trials are warranted to show the performance in ovarian cancer detection.

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