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Screening for ovarian cancer: imaging challenges and opportunities for improvement

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Abstract

The United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) recently reported a reduction in the average overall mortality among ovarian cancer patients screened with an annual sequential, multimodal strategy that tracked biomarker CA125 over time, where increasing serum CA125 levels prompted ultrasound. However, multiple cases were documented wherein serum CA125 levels were rising, but ultrasound screens were normal, thus delaying surgical intervention. A significant factor which could contribute to false negatives is that many aggressive ovarian cancers are believed to arise from epithelial cells on the fimbriae of the fallopian tubes, which are not readily imaged. Moreover, because only a fraction of metastatic tumors may reach a sonographically-detectable size before they metastasize, annual screening with ultrasound may fail to detect a large fraction of early-stage ovarian cancers. The ability to detect ovarian carcinomas before they metastasize is critical and future efforts towards improving screening should focus on identifying unique features specific to aggressive, early-stage tumors, as well as improving imaging sensitivity to allow for detection of tubal lesions. Implementation of a three-stage multimodal screening strategy in which a third modality is employed in cases where the first-line blood-based assay is positive and the second-line ultrasound exam is negative may also prove fruitful in detecting early-stage cases missed by ultrasound.

Keywords

Ovarian cancer screening; ultrasound; multimodal screening; CA125; fallopian tube; UKCTOCS; PLCO

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Introduction

When ovarian cancer is detected at an early stage (I or II), cytoreductive surgery and conventional chemotherapy can cure 70–90% of patients, compared with approximately 20% or less of those patients diagnosed at late stage (III and IV).[1] At present, only 20–25% of ovarian cancers are diagnosed in early stage I/II. Because detecting cancer at an early, localized stage typically yields better surgical outcome, which is currently the most effective treatment, early staging is critical. Even aggressive high-grade, poorly or undifferentiated tumors have a much higher 5-year survival rate when diagnosed in stage I/II versus III/IV (74% vs. 27%).[2] In light of these statistics, which suggest that earlier detection may convey a survival advantage, efforts have been made to develop effective ovarian cancer screening strategies. While prophylactic bilateral salpingectomy or salpingo-oophorectomy may be an option for reducing risk among high-risk women, it is inappropriate for normal-risk women, who represent 75 – 85% of ovarian cancer cases.[3–5] Thus, we conducted a critical review of current ovarian cancer screening approaches, focusing on the role of ultrasound, including its strengths and weaknesses, as well as additional strategies for potentially improving screening in the future, such as implementation of a three-stage multimodal approach.

Ovarian cancer biology

Ovarian masses are pathologically classified as benign, malignant (invasive), or of low malignant potential (borderline). Borderline tumors, which have markedly atypical histology but do not invade the basement membrane underlying the epithelium, account for approximately 15% of epithelial ovarian tumors and generally have a much better prognosis than invasive ovarian tumors, with 10-year survival rates ranging from 85% to 98% depending on histologic type, disease stage, and patient age.[6, 7]

Among invasive ovarian cancers there are two major subtypes, often classified as Type I or Type II tumors.[8, 9] Type I tumors typically present in early stage and are clinically indolent; Type I tumors include low-grade serous carcinomas, low-grade endometrioid carcinomas, mucinous carcinomas, clear cell carcinomas, and malignant Brenner (transitional) tumors. Type II tumors account for approximately 75% of epithelial ovarian cancers and include high-grade serous carcinomas, high-grade endometrioid carcinomas, undifferentiated carcinomas, and malignant mixed mesodermal tumors. High-grade serous carcinoma is the most common morphology, accounting for 50 – 70% of invasive ovarian cancers. Most, if not all, Type II tumors have TP53 mutations, are very aggressive, and typically present in late stage.[10, 11] Because Type I tumors are generally less aggressive, they are more likely to grow slowly to a large size while remaining within the ovaries, and thus are more likely to be detected in early stage. On the other hand, aggressive Type II ovarian tumors often metastasize before detection, with stage I high-grade serous ovarian cancer representing only 1% of primary ovarian cancer diagnoses using conventional methods.[12, 13] A retrospective chart review comparing the average size of primary ovarian tumors across early-stage (I/II) and late-stage (III/IV) patients further supports the theory of two distinct ovarian cancer subtypes by revealing significantly larger sizes among early-stage cases (10.7 cm versus 4.8 cm, respectively).[14]

Type I tumors are believed to originate from precursor lesions in the ovary (e.g., endometriosis or borderline tumors), while Type II tumors are thought to develop *de novo* from the ovarian surface epithelium, subserosal inclusion cysts, or from the fimbriae of the fallopian tubes.[15–17] Mouse models support development of high-grade serous ovarian cancer from both the fallopian tubes and ovaries.[18–20] Samples collected during prophylactic salpingo-oophorectomies performed on women with BRCA1 or BRCA2 germ line mutations have revealed tubal involvement in an estimated 76% of early gynecologic malignancies.[21–26] As 10 – 15% of invasive ovarian cancers arise in BRCA1/2 mutation carriers, at least 10% of all ovarian cancers arise from the fallopian tube.[27, 28] When combining this statistic with sporadic (non-familial) high-grade serous carcinomas that coat the ovary rather than growing from the surface and likely arise from the fallopian tube, which represents approximately 20% of total cases, at least 30% of ovarian cancers may originate from the fallopian tube.[29–31] Examination of fallopian tube specimens has revealed high expression of p53 and clonality between serous tubal intraepithelial carcinoma and high-grade serous ovarian carcinomas.[32–35] Cells in the distal region of the fallopian tubes are likely more prone to malignant transformation due to pro-inflammatory microenvironmental factors associated with ovulation, as well as the relatively large surface area of the fimbria. Once serous tubal intraepithelial carcinoma develops, these malignant cells are believed to migrate onto the nearby ovarian surface and/or the surrounding peritoneum. Metastatic cells are transported through the peritoneal fluid and implant on the surface of the omentum or the visceral or parietal peritoneum, which provide a favorable microenviroment for cancer cells to grow.[36–38]

Background on ovarian cancer screening

Past clinical trials

Both primary ultrasound screening and multimodal strategies incorporating ultrasound have been evaluated for ovarian cancer detection during several large-scale clinical trials. The University of Kentucky Ovarian Cancer Screening Project annually screened 37,293 women between 1987 and 2011 with primary transvaginal ultrasound (TVU); to reduce false positives, measurements of the serum biomarker CA125 were also taken into account after detection of a pelvic mass.[39] A non-sequential, multimodal strategy, which employed annual TVU scans and annual screening for single elevated values of serum CA125 was evaluated in the Prostate, Lung, Colorectal and Ovarian (PLCO) Cancer Screening Trial. The PLCO trial recruited 78,216 women between the ages of 55 and 74 to undergo either annual ovarian cancer screening (n = 39,105) or to receive conventional care (n = 39,111).[40] Because the two screening modalities were conducted independently and not used in combination, referral to a gynecologist resulted either from an abnormal TVU scan or from an elevated CA125 measurement. The United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS), which enrolled postmenopausal women at average risk of ovarian cancer, evaluated both a sequential, multimodal arm and a primary ultrasound arm.[41] In the UKCTOCS, 101,359 women received conventional care as controls, 50,639 underwent annual ultrasound, and 50,640 underwent annual CA125 measurements, which were analyzed using a risk of ovarian cancer algorithm (ROCA)[42] where rising CA125 levels,

even if within the normal range, prompted ultrasound. In the multimodal screening arm, rising CA125 levels prompted TVU in approximately 2% of participants each year.

Criterion for success

For a screening strategy to be considered effective, it must achieve a sufficient positive predictive value (PPV). Achieving a high PPV is critical for reducing unnecessary operations and the risk of surgery-related complications in otherwise healthy women. In the UKCTOCS, 3.4% false-positive screens (i.e., women who underwent surgery and were diagnosed with normal or benign pathology) experienced surgery-related complications (e.g., hollow viscus injury, hemorrhage, hernia, wound breakdown, infection, etc.).[43] In the PLCO trial, a much higher surgery-related complication rate of 15% was reported across screened women who had normal or benign pathology (false-positive screens).[44] As one might expect, in both the UKCTOCS and PLCO trials, more women underwent unnecessary operations in the screening group compared with the usual care group.[43, 44] A consensus has developed that a minimum PPV of 10% will be required, where no more than 10 operations are performed for each ovarian cancer diagnosis.[45] In addition to having high enough sensitivity and specificity to achieve an adequate PPV, an effective strategy must also reduce mortality in a cost-effective manner.[46]

Trials outcomes

The University of Kentucky Ovarian Cancer Screening Project reported a PPV of 14.5% and a statistically significant stage shift with 70% of screen-detected invasive epithelial ovarian cancers in early stage I or II.[39] Across all screen-detected invasive epithelial ovarian cancers, the 5-year survival rate was 74.8% ± 6.6% compared with 53.7% ± 2.3% for unscreened patients treated at the same facility following the same clinical practices. Because survival rates, which are subject to lead-time bias, were reported rather than mortality, these results do not, however, prove that screening reduced deaths from the disease and the perceived survival benefit may instead be attributed to an earlier diagnosis of disease with no impact on lifespan.

Early results from the prevalence phase of the UKCTOCS were encouraging, suggesting that sequential, multimodal screening (i.e., CA125 with ROCA followed by ultrasound) was able to detect 89.5% of all primary invasive epithelial ovarian and tubal cancers with a specificity of 99.8% and a PPV of 35.1% (i.e., three operations for each ovarian cancer diagnosis).[41] Furthermore, 47% of prevalent ovarian cancers detected through screening were in early stage. A smaller study of 4,675 women coordinated by the MD Anderson SPORE in Ovarian Cancer, which utilized the same screening approach as the multimodal arm of the UKCTOCS, reported a similar specificity of 99.9% and a PPV of 40%.[47] Mortality results from the UKCTOCS were also encouraging with a significant reduction of 20% in the average overall mortality among patients who underwent sequential, multimodal screening (excluding prevalent cases and primary peritoneal disease); however, given the broad confidence limits around the estimate of reduced mortality, additional follow-up is needed before final conclusions can be formed on the survival advantage of such screening.[43] Comparison of results from the primary ultrasound and multimodal screening arms of the UKCTOCS revealed a lower sensitivity for detecting invasive epithelial ovarian and

fallopian tube cancers diagnosed within one year of screening, a lower percentage of early-stage (I/II) invasive ovarian, tubal, and undesignated cancers, and a lower PPV for detecting invasive ovarian and tubal cancers in the ultrasound screening arm (66%, 23%, and 5% vs. 87%, 38%, and 23%, respectively).[43] Furthermore, the ultrasound arm exhibited a smaller mortality reduction of 11 – 12% (including prevalent cases) compared with 15 – 16% in the multimodal arm.

In contrast to the promising results obtained in the UKCTOCS, which suggest a high PPV, a shift to earlier stage diagnoses, and a possible survival benefit, the screening strategy evaluated in the PLCO failed to improve survival or to detect an increased fraction of early-stage ovarian cancer cases.[44] Additionally, the PPV for detecting invasive ovarian cancers was much lower in the first four annual screening rounds of the PLCO than in the UKCTOCS at 2.6% or 0.9% for women with a positive CA125 or TVU screens, respectively.[48] In retrospect, the PPV would have risen to 20% if both CA125 and TVU screens were positive, although over 80% of cases would have been missed. Possible explanations for the discrepancy between the PLCO and the UKCTOCS could be that a non-sequential approach was used in the PLCO (i.e., TVU and CA125 screens were conducted independently of one another). Additionally, the PLCO trial employed a fixed cutoff for CA125, whereas the UKCTOCS utilized each woman's own baseline and prompted referral for TVU even among women with CA125 levels below an arbitrary cutoff defined for the general population. Furthermore, in the UKCTOCS, women with a modest rise in CA125 and an intermediate risk of developing ovarian cancer were retested 3 months later, while women returned after one year in the PLCO trial. Another explanation for the absence of a mortality benefit in the PLCO trial could lie in the lack of a protocol for managing patients with positive screens. Management was instead left to the discretion of each patient's physician without a clear policy for follow-up and surgical exploration.[49] Because prompt surgical intervention, optimal cytoreductive surgery, and effective chemotherapy each impact survival, if physicians were unsure as to how to manage a positive screen or preferred to take a watchful waiting approach, a long lapse between an initial positive screen and surgery could result, negating the expected benefit of screening and minimizing the chance to produce a stage shift.

While the inclusion of specified protocols mandating patient management following positive and negative screens was a strength of the UKCTOCS, one potential disadvantage experienced by some patients in the multimodal arm was an increased time to surgical intervention given the need for repeat testing.[41] Among women in the multimodal arm who were diagnosed with ovarian or tubal malignancies during the prevalence phase of the UKCTOCS, 21% were characterized as intermediate risk during the level 1 screen, which called for additional follow-up testing, extending the median time to surgery to 273.9 days. On the other hand, a different protocol for triaging patients was followed for the ultrasound arm and the median time from the level 1 scan to surgery was 81.5 days for screen-detected ovarian or tubal malignancies.[41] Although early-stage ovarian tumors are believed to double every 4 months, the relevance of an extended time from detection to surgery and its effect (if any) on lifespan is unknown in this case.[50]

Benefits of ultrasound inclusion in screening for ovarian cancer

TVU or TVS (transvaginal sonography) is generally accepted as the primary imaging modality for evaluation of an adnexal mass and has largely replaced transabdominal ultrasound given its detailed anatomic depiction of pelvic anatomy, superior resolution, and better performance in obese patients.[51–53] TVU is an attractive screening modality because it is safe, cost effective, and well tolerated by patients. When included as part of a multimodal ovarian cancer screening strategy based on serum biomarkers (e.g., CA125), the primary benefit of ultrasound is to reduce false positives and the number of unnecessary operations. In addition to ruling out false positives, the inclusion of ultrasound can also be useful in diagnosing cases where expression of CA125 is weak or absent, which has been shown to be true in up to 22% of ovarian cancer tissue samples.[54]

Specificity

Some benign ovarian and gynecologic conditions (e.g., benign ovarian cysts, endometriosis, leiomyoma, etc.) cause elevated serum CA125 levels, resulting in false positives.[55, 56] Thus, the PPV of serum CA125 is insufficient to warrant its use as a stand-alone ovarian cancer screening modality. In the first four screening rounds of the PLCO, a PPV of 2.6% was achieved when using single serum CA125 levels at or above a fixed threshold of 35 U/mL, while a much higher PPV of 20% was reported when positive CA125 and TVU results were retrospectively combined.[48] As an advancement over using a fixed CA125 cutoff, applying the ROCA algorithm to CA125 measurements serially collected from over 9,000 women at Royal London and St. Bartholomew's Hospitals achieved a PPV of 15%.[42] Despite showing improvement over a threshold approach, the PPV of the ROCA approach is still inferior to that of multimodal screening (ROCA followed by TVU), which reported a 23% PPV in the UKCTOCS.[43]

Physician confidence

In addition to improving the PPV of ovarian cancer screening, another potential benefit of TVU inclusion is that physicians may be more confident and trusting of abnormal TVU results given that physicians in the PLCO trial were more likely to refer to surgery based on a positive ultrasound screen than a positive serum CA125 screen. In four rounds of annual screening in the PLCO trial, the surgery rate following a positive TVU screen was 27.2%, while the surgery rate following a positive CA125 screen was 11.7%; of note, while the overall surgery rates among those with positive screens dropped after the first year, it remained higher for TVU than CA125 throughout all years.[48]

Sensitivity

TVU enables high-resolution imaging of the ovaries and is especially useful in distinguishing simple cysts from complex cystic masses and solid tumors, which is important given the ultrasound characteristics of histologically-proven adnexal malignancies. Because the features indicative of malignancy, including cystic lesions with a large solid component, thick wall and/or septations greater than 3 mm, mural nodules, and necrosis of a solid component/mass, are analogous for TVU and cross-sectional imaging modalities (i.e., computed tomography (CT) and magnetic resonance imaging (MRI)), these

modalities offer limited additional value for ovarian cancer screening. Furthermore, the spatial resolution of ultrasound generally exceeds that of CT and MRI in diagnosing small lesions. Because CT offers comparable ability to detect malignancy, yet has a higher cost and exposes patients to ionizing radiation, it is not recommended for ovarian cancer screening.[57] Positron emission tomography (PET) with CT (PET/CT) is also not recommended for ovarian cancer screening since it has a relatively low spatial resolution, which hinders the detection of small tumors, and involves exposure to ionizing radiation. Additional challenges of using PET/CT include physiologic uptake in normal structures, such as ovarian follicles in late follicular to early luteal phase in premenopausal women, adjacent bowel mucosa, excretion into the bladder, which may obscure small pelvic malignancies, as well as other confounding conditions that may lead to false positive (inflammation, endometriosis, pedunculated leiomyomas) and false negative (early adenocarcinomas, borderline/low-grade tumors) results.[57] A wide range in sensitivity and specificity (58–100% and 67–92%, respectively) has been reported for the detection of ovarian malignancies in women with adnexal masses using PET or PET/CT.[58–61] While the sensitivity and spatial resolution of ultrasound is generally better or comparable to other imaging modalities, MRI has reported greater accuracy and specificity in the diagnosis of malignant adnexal masses (89% and 84%, respectively, versus 64% and 40%).[62] However, because of its relatively high cost and lower availability, MRI is not typically the first-line imaging modality for the evaluation of adnexal masses.

Current challenges facing ultrasound in the screening setting

While imaging is the focus of this review, it is important to note that the sensitivity of a sequential, multimodal ovarian cancer screening method, such as the one evaluated in the UKCTOCS, is initially limited by the sensitivity of the first-line test (e.g., serum CA125). As such, the overall mortality benefit and cost effectiveness of screening is highly dependent upon the first-line screen.[63] That being said, second-line screening has the potential to further limit sensitivity and the lower sensitivity reported in the ultrasound arm of the UKCTOCS compared with the multimodal arm suggests that TVU may hinder early detection. In addition to reporting lower sensitivity in the ultrasound arm, multiple cases of women with ovarian cancer who had rising serum CA125 levels but normal ultrasound screens were documented in the multimodal arm. The biology of ovarian cancer, which is believed to consist of two primary disease sub-types, poses an obstacle in that annual screening with TVU is expected to be more effective in detecting the more indolent, Type I tumors in early stage.[39, 64] Additionally, a significant fraction of high-grade serous ovarian cancers are believed to originate in the fimbriae of the fallopian tubes as very small tumors before the cancer progresses to an advanced stage, yet there is little to no documented experience in imaging this anatomy.

False negatives

In the UKCTOCS, there were multiple cases where the serum CA125 levels were rising, but TVU was normal in women later diagnosed with ovarian cancer (i.e., false negatives). Model-based estimates indicate that the minimum tumor size necessary to secrete enough CA125 to generate a positive biomarker screen (i.e., serum CA125 > 34.11 U/mL) is 116.7

mm^3, which corresponds to a 3 mm diameter spherical tumor.[65] Based on this estimate, it is unlikely that tumors smaller than 3 mm in diameter would be detected when using serum CA125 with a fixed cut-off of 34.11 U/mL as a first-line screen; however, ROCA (rather than a fixed cut-off) was used in the UKCTOCS and multiple patients with serum CA125 levels below 35 U/mL were diagnosed with ovarian cancer. Thus, while the minimum tumor diameter necessary to generate a positive screen with ROCA is unknown, it may be possible to detect tumors smaller than 3 mm. Detecting very small tumors with TVU presents a challenge and when considering those patients in the UKCTOCS diagnosed with invasive epithelial ovarian or tubal cancers that were flagged by ROCA but had CA125 levels below 35 U/mL, 41% showed no abnormality on the initial TVU scan, possibly because their tumor volume was small given that 49% of these patients were diagnosed at an early stage (I or II).[66] As a result of the need for repeat testing, the interval from screening to surgery was significantly longer for the low CA125 patients as compared with patients who had CA125 levels above a 35 U/mL threshold (30 versus 12 weeks, respectively). Thus, there is a need for more sensitive imaging in those patients with positive biomarker screens, and it has been suggested that improved imaging is the key to a successful ovarian cancer screening program.[67]

Computer-based models estimate the median diameter of early-stage serous ovarian tumors to be less than 3 mm in BRCA-positive women and, during the 4.3 years that these tumors are estimated to persist in early stage, it is believed that they are typically smaller than 9 mm in diameter for an average of over 3.8 of those years.[50] Thus, while there may be a short window of opportunity for TVU detection of early-stage, Type II tumors which have grown to a detectable size but remain localized, this window may be missed with annual, and even semi-annual, TVU screening.[68] As a further challenge, data collected during prophylactic salpingo-oophorectomies in patients with BRCA1/2 mutations have revealed primary tumor diameters smaller than 10 mm across multiple late stage (III/IV) patients.[21, 69] Thus, some tumors may metastasize before ever reaching a size that is detectable by TVU. TVU also often fails to detect tumors in women with a normal ovarian volume, such as primary peritoneal cancers that involve the ovarian surface and do not cause ovarian enlargement. Several studies of women at high risk have revealed minimal to no sonographic abnormalities in patients with high-grade serous ovarian cancer despite many being in advanced disease stage.[70, 71] Among false-negative ultrasound screens in the University of Kentucky Ovarian Cancer Screening Project (i.e., women diagnosed with ovarian cancer within 12 months of a negative screen), 33% (3 out of 9) had normal-sized ovaries with extra-ovarian metastases at the time of surgery and 78% were stage III.[72] Likewise, in the prevalence phase of the UKCTOCS, all false negatives reported in the ultrasound screening arm were in late stage (III/IV). In the PLCO trial, 85% of screen-detected ovarian cancers with negative TVU screens were detected in late stage III/IV and 54% of fatal screen-detected cases had negative TVU screens (not including those without TVU results).[73] A lower five-year survival rate among CA125-positive women in the PLCO with negative TVU screens (42% vs. 67% for women with positive TVU screens) could indicate that TVU is inadequate for detecting more aggressive, fatal cancers.[73]

False positives

The overlap in sonographic features between early cancer and some benign lesions represents another limitation of ultrasound. The low PPV observed in the ultrasound screening arm of the UKCTOCS compared with the multimodal arm (5% versus 23%, respectively) illustrates the relatively high false-positive rate of ultrasound for the evaluation of adnexal masses;[43] an even lower PPV of 0.9% based on an abnormal TVU screen was reported in the first four rounds of the PLCO trial.[48] In both the PLCO and The University of Kentucky Ovarian Cancer Screening Project, primary borderline epithelial neoplasms of the ovary were classified as false positives since they are of low malignant potential and are associated with lower morality rates. However, these borderline tumors were considered true positives in the UKCTOCS and the majority of screen-detected borderline cases (67%) were from the ultrasound arm.[43] Of all primary ovarian cancers detected through screening, 30% were borderline in the ultrasound arm, while only 12% of those detected through multimodal screening were borderline. Furthermore, 91% of borderline tumors were detected through screening in the ultrasound arm compared with 55% in the multimodal arm. Similarly, in the initial screen of the PLCO trial, all nine non-invasive cystadenomas of low malignant potential (borderline cases) were detected through TVU.[40] In addition to detecting more borderline tumors, unpublished data from the UKCTOCS also indicates that ultrasound is more sensitive for detecting Type I versus Type II tumors.[74] Because borderline and Type I ovarian tumors have a much lower incidence of mortality compared with Type II tumors, detecting an increased fraction of these cases is unlikely to have a substantial impact on mortality. Therefore, while ultrasound is capable of detecting clinically-indolent lesions in early stage, such detection may reflect overdiagnosis, or the detection of disease that would not ultimately cause mortality.

Operator dependence

A well-documented limitation of ultrasound is operator dependence.[75] While new equipment and transducers have made TVU much simpler to operate, inter-observer variation still exists due to a lack of sufficient experience or to a misunderstanding of ovarian physiological anatomy. Sonologists will likely only gain the necessary experience to improve performance by performing large numbers of exams, seeing the same patient repeatedly in a screening setting, and comparing current to prior exams, while consistently ensuring meticulous technique.

Non-visualization

In the ultrasound arm of the UKCTOCS, 6.2% or 3005 women required a repeat scan following an unsatisfactory scan in which one or both ovaries could not be visualized while also not achieving a good view of the iliac vessels.[41] Common reasons for non-visualization of the ovaries by TVU include obesity, previous gynecological surgery (hysterectomy, unilateral oophorectomy, and tubal ligation), increasing age, presumably due to small atrophic ovaries, and ovaries located superiorly out of the range of TVU probe.[76] When the ovaries cannot be visualized with TVU, transabdominal ultrasound should be attempted, although it is unlikely to improve visualization in obese patients.

Opportunities for improvement

First-line screening

The inclusion of additional blood-based biomarkers besides CA125 (e.g., HE4 or CA72-4) could further enhance the sensitivity of first-line screening for detecting early-stage ovarian cancer.[77–80] Techniques using proteomic profiles as biomarkers have also been developed for screening, but have not yet proven sufficiently sensitive or reproducible to be used clinically.[81, 82] Another potential means of improving first-line screening may come through liquid biopsies, which detect circulating tumor DNA (ctDNA) mutations, circulating tumor cells (CTCs), elevations in the overall level of cell-free DNA (cfDNA), and DNA methylation or other epigenetic biomarkers in extracted bodily fluids.[83–91] Liquid biopsies based on ctDNA, as compared with CTCs, may offer greater sensitivity for early detection in light of some evidence suggesting a higher yield and frequency of ctDNA in liquid biopsy samples.[92] Studies detecting ctDNA in ovarian cancer patients in the form of genetic alterations have reported a 41% (9/22) detection rate of known mutations using routine liquid Pap smear samples,[93] a 93% (28/30) detection rate of tumor-specific p53 sequence in the peritoneal wash fluid,[94] and an 80% (8/10) detection rate for tumor-specific chromosomal junctions in plasma samples.[95] Because the aforementioned studies mainly included late-stage patients, the utility of these assays for detecting early-stage disease is uncertain. Additionally, the sensitivity of such assays, which are often performed using polymerase chain reaction techniques and thus have an inherent sensitivity limit of 0.01%, may limit their use for early detection given that ctDNA only represents a fraction of total DNA in the sample.[96] While evidence of detectable concentrations of ctDNA has been reported among patients with localized disease, indicating that such liquid biopsies may prove useful for detecting early-stage cases, there is currently little to no prospective evaluation of their utility in a screening setting.[92, 97] Furthermore, large variability in the concentration of ctDNA in liquid biopsy samples exists across patients even at the same disease stage (e.g., ctDNA concentrations varying from < 1% to > 40% have been reported among late-stage (III/IV) high-grade serous ovarian cancer patients),[95] despite the assumption that concentration is related to tumor burden. Further study is needed to characterize this variability, as well as the relationship between disease extent and ctDNA concentration to determine if early detection is viable. Additional limitations to the use of liquid biopsies in the screening setting are the time and cost currently required to perform such assays, as well as the potential for a lack of specificity, as in the case of screening for *TP53* mutations which have also been observed in healthy patients.[98] Because such screening may not offer complete specificity and also may not provide insight to the location of disease when a mutation common to several potential disease sites is identified (e.g., *TP53*), the need for additional follow-up with imaging or another method remains. However, a means of establishing specificity, as well as localizing the disease may not exist if the sensitivity of liquid biopsies or autoantibody serum biomarkers surpasses that of traditional imaging modalities, thus justifying the need for more sensitive second-line imaging techniques.

Second-line screening

Ultrasound technique refinement—Improving the performance and consistency of ultrasound screening is important for ensuring diagnostic accuracy, as well as reducing the need for repeat scanning due to unsatisfactory exams. One means of improving screening is through the development and implementation of quality assurance procedures, which led to higher visualization rates of the ovaries among enrolled centers over the course of the UKCTOCS; this trend was noted even among experienced sonographers (those who had performed >1000 UKCTOCS screens).[99] In addition to implementing quality assurance procedures, published guidelines and standards should also be followed. For instance, existing guidelines from the Society of Radiologists in Ultrasound should be utilized when evaluating asymptomatic cysts.[100] Following such guidelines would help physicians to recognize that many postmenopausal women have ovarian cysts, often exceeding 3 – 4 cm, which require only annual or biannual surveillance and not intervention. Furthermore, strict criteria for malignancy should be applied to complex masses, where mural nodularity with increased blood flow is the most important factor, and hemorrhagic material should not be mistaken for septations.

To aid in differentiation of benign versus malignant tumors, specific criteria based on ultrasound characteristics have been developed by the International Ovarian Tumor Analysis (IOTA) group to improve specificity;[101] however, this criteria has been shown to generate a relatively higher number of inconclusive results among postmenopausal women.[102–104] A review of the ultrasound characteristics of histologically-proven adnexal malignancies was conducted across a subgroup of patients from the IOTA study to classify sonographic features by disease sub-type. This analysis revealed common features among borderline and stage I primary invasive ovarian epithelial cancers (nearly half of which [20 out of 42] were grade 1) relative to stages II, III, and IV primary invasive ovarian epithelial cancers, including a larger volume, a larger fraction with papillary projections, a larger fraction of multilocular cysts without solid components, and a smaller proportion of purely solid tumors.[105] However, the small number of high-grade, early-stage cases documented with TVU reflects our present inability to distinguish unique sonographic characteristics among this disease sub-type. The identification of common features in later stage tumors, such the appearance of a complex (cystic and solid) mass, which typically corresponds to ovarian cancer beyond stage II, will not lead to earlier detection but may help to reduce false positives.

While screening is typically performed on a postmenopausal patient population, annual surveillance may also be performed on premenopausal women at high risk for ovarian cancer. Additionally, some women who are clinically labeled as postmenopausal have cyclically-changing ovarian cysts and would be more accurately described as perimenopausal. In premenopausal and perimenopausal women, false positives are commonly due to misdiagnosis of physiologic cysts (e.g., corpus luteum or hemorrhagic follicular cysts) as potentially invasive cancers. In these benign cysts, hemorrhage and mural irregularity, as well as proliferation of blood vessels at the margins, is often mistaken for the complex nodularity and neovascularity of early cancer. In such cases, the impression of possible malignancy leads to further diagnostic work-up and/or surgical intervention in

patients without ovarian cancer (i.e., false positives). For this reason, ultrasound exams of high-risk premenopausal women should be performed in the first ten days of a new menstrual cycle to avoid the corpus luteum.

When a questionable lesion arises, a follow-up ultrasound exam should be performed six to eight weeks after the original scan rather than diagnosing possible malignancy. This has the benefit of not only definitively resolving the indeterminate lesion and compensating for prior ultrasound exams of suboptimal technique, it avoids false-positive diagnoses made by operators who are relatively inexperienced in gynecologic ultrasound. To further reduce false positives and negatives, a radiologist, gynecologist, or other recognized expert should personally scan the patient, ensuring optimal technique. Such strategies will result in more accurate interpretation of examinations, reduce inter-observer variation, and will likely improve the overall performance of ultrasound imaging in the screening setting.

Doppler ultrasound—An investigational ultrasound technique is the examination of ovarian blood flow by Doppler ultrasound. Retrospective examination of ultrasound studies have resulted in reports of early-stage ovarian cancers accompanied by the presence of abnormal central ovarian blood flow, distinct from the expected hilar or normal peripheral flow.[106, 107] Patient studies have also revealed such features (i.e., central blood flow, as well as low resistive and pulsatility indices) to be associated with ovarian malignancies, although not in the setting of ovarian cancer screening.[108–116]

Microbubble enhancement—Microbubble contrast-enhanced TVU also studies increased blood flow in an unexpected clinical setting, such as a postmenopausal ovary, and has shown promise for differentiating benign from malignant adnexal masses.[117–121] Although microbubble enhancement holds promise for improving specificity, it may not improve detection of early-stage disease. In the case of tumors localized to the fallopian tubes, anatomical demonstration of the tubes by conventional ultrasound is necessary or microbubble examination will be a challenge.

Integration with photoacoustic imaging—One emerging technology that can be co-registered with ultrasound is photoacoustic imaging, which allows for high-resolution detection of angiogenesis, and thus has the potential to detect active neovascularization in early-stage ovarian tumors.[122, 123] Shortcomings of this approach include a limited tissue penetration depth of approximately 5-cm and a decline in spatial resolution with increasing depth; however, these limitations may be overcome through integration with TVU.[124, 125] Promising results have been obtained through *ex vivo* analysis of postmenopausal human ovaries using co-registered ultrasound and photoacoustic imaging to quantify light absorption, which is related to vascular density and distribution, resulting in accurate classification of 83% of cases (10 of 12) as normal or malignant.[126] While similar results have been reported by others, sufficient *in vivo* human validation data is lacking.[127–129] The administration of gold nanorod contrast agents has been shown to enhance the photoacoustic signal, as well as visualization of tumor margins in ovarian cancer mouse models, and could be beneficial for future human use.[130]

Third-line screening

Another possible means of improving screening would be to include an additional third-line modality in patients with negative ultrasound scans preceded by positive biomarker screens. While third-line screening modalities are likely to be more expensive than TVU, the number of patients receiving such screening would be very small (e.g., < 1% of patients during the prevalence screen of the UKCTOCS),[41] and so this would not be expected to have a substantial impact on the overall cost effectiveness of a multimodal ovarian cancer screening program.

Conventional imaging modalities—Given its high specificity, MRI is a useful follow-up modality for discriminating sonographically-indeterminate adnexal masses and the combination of ultrasound, MRI, and FDG-PET has been shown to further improve specificity.[58, 59, 131–133] However, because ultrasound on its own generally provides high sensitivity and spatial resolution, including additional established imaging modalities is only likely to improve specificity and PPV of screening by better distinguishing between malignant and benign masses and is unlikely to improve sensitivity or to allow for detection of lesions at an earlier stage, and thus unlikely to impact disease-specific mortality.

Hyperpolarized MRI—An experimental technique that may offer improved sensitivity over TVU is hyperpolarized MRI. Hyperpolarized [1-^{13}C]pyruvate can be used as an imaging tracer to characterize cancer metabolism and has been reported to generate a signal in the pre-tumors of mouse models prior to the formation of a primary tumor.[134] Hyperpolarized ^{13}C MRI has shown a unique signature within the tumor of prostate cancer patients and could potentially be applied for ovarian cancer as well.[135]

Magnetic relaxometry—Another promising technology for improving sensitivity in multimodal screening is magnetorelaxometry or magnetic relaxometry (MRX).[136, 137] MRX detects binding between targeted iron oxide nanoparticles and cancer cells or tumor vessels and has been utilized for a variety of applications, including breast cancer detection in mice, detecting minimal residual disease in leukemia patient bone marrow biopsies, and quantifying nanoparticle accumulation in biological samples.[136–144] Studies indicate that 10^6 ovarian cancer cells bound to superparamagnetic iron oxide nanoparticles conjugated with anti-ovarian cancer associated antigens can be detected.[145, 146] The application of MRX would be to detect, though not image, small cancers of the fallopian tube or ovary and, while the expected sensitivity is greater than that of TVU, it has not achieved clinical translation.

Conclusion

The number of unnecessary operations resulting from ovarian cancer screening has been greatly reduced by using screening protocols incorporating CA125 with ROCA followed by ultrasound, as reflected by the high PPV reported in the multimodal arm of the UKCTOCS and an analogous trial performed at MD Anderson Cancer Center.[47] Moreover, a 20% mortality reduction was achieved in the multimodal arm of the UKCTOCS, albeit with broad confidence limits around that estimate. While progress toward improving ovarian cancer screening has been made, there is opportunity for further improvement. The results of this

review suggest that such opportunities lie in improving the sensitivity of the second-stage of screening or in implementation of a third-line screen, assuming that biomarkers are sensitive enough to detect early-stage cases. Future efforts towards improving screening should focus on detecting aggressive, Type II ovarian tumors prior to metastasis.

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