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GYNECOLOGIC CANCERS (NS REED, SECTION EDITOR)

# Novel Approaches to Ovarian Cancer Screening

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## Abstract

**Purpose of Review** Both conventional and novel approaches to early detection of ovarian cancer are reviewed in the context of new developments in our understanding of ovarian cancer biology.

**Recent Findings** While CA125 as a single value lacks adequate specificity or sensitivity for screening, large studies have shown that a 2-stage strategy which tracks CA125 change over time and prompts transvaginal ultrasound (TVS) for a small subset of women with abnormally rising biomarker values achieves adequate specificity and detects a higher fraction of early-stage disease. Sensitivity could clearly be improved in both blood tests and in imaging. Metastasis can occur from ovarian cancers too small to increase blood levels of protein antigens and a significant fraction of ovarian cancers arise from the fimbriae of fallopian tubes that cannot be imaged with TVS. Autoantibodies, miRNA, ctDNA, DNA methylation in blood, and cervical mucus might improve sensitivity of the initial phase and magnetic relaxometry and autofluorescence could improve imaging in the second phase.

**Summary** Enhancing the sensitivity of two-stage strategies for early detection could reduce mortality from ovarian cancer.

**Keywords** CA125 · Ovarian cancer screening · Novel approaches · HE4 · Fallopian tube cytology · TP53 · miRNA · ctDNA · Autoantibodies · Ovarian cancer screening trials · Biomarkers · DNA methylation · Transvaginal ultrasound · Fallopian tube theory of carcinogenesis

## Introduction to Ovarian Cancer

The lifetime risk of ovarian cancer is approximately 1.3% in the general population; however, it has the highest mortality of all gynecologic malignancies [1]. Ovarian cancer incidence increases with age, particularly after age 45, with the median age at diagnosis of 63 years [2, 3]. In 2018, there were approximately 22,240 new cases of ovarian cancer and 14,070 deaths in the USA [4], with 295,414 new cases and 184,799 deaths worldwide [5]. Ovarian cancer is the fifth leading cause of cancer deaths among US women and the eighth leading cause of death among women worldwide [6].

If ovarian cancer is detected early, the 5-year survival rates are 90% if confined to the ovary (stage I) or 70% if confined to the pelvis (stage II). However, most ovarian cancer are diagnosed at stages III (51%) and IV (29%) [1], when 5-year survival rates are less than 30% [1, 7]. Overall 5-year survival ranges between 30 and 40% worldwide, and has increased little (2–4%) over the last two decades [8]. Additionally, 70% of patients with advanced epithelial ovarian cancer will have cancer recurrence, after which time survival is extremely low.

## Biology of Ovarian Cancer

Ovarian cancers are divided into epithelial ovarian cancers (EOCs), which are the most common type and make up 90% of cases, and non-epithelial cancers representing only

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10% of cases. Epithelial ovarian cancers arise from the simple flattened surface epithelial cells that cover the ovary, subserosal inclusion cysts, and/or the fimbriated end of the fallopian tubes [9, 10]. These epithelial cells transform into different histotypes including cells that resemble the lining of the fallopian tube (serous, 52%), endometrium (endometrioid, 10%), endocervical glands (mucinous, 6%), or vaginal rest cells (clear cells, 6%); unspecified or rare histotypes account for the remaining quarter of EOCs [1]. EOCs are further classified into type I and type II based on clinical and pathologic features. Type I EOCs are usually low-grade tumors that are diagnosed in early stages (I/II) and show slow growth with low mortality including low-grade serous and endometrioid. Type I EOCs usually develop from benign lesions, like endometriosis, that implant into the ovary and through a series of mutations result in malignant transformation [10, 11].

Type II EOCs are usually high grade, present at later stages (III/IV), and include high-grade serous, carcinosarcoma, and undifferentiated histotypes. They show aggressive growth and have low survival rates. Most type II EOCs are associated with *TP53* mutations. In addition to the pelvic tumor, the tumor growth is usually abundant in the omentum and mesentery [10–13].

Non-epithelial ovarian cancers (10%) include germ cell tumors, sex cord-stromal tumors, ovarian sarcoma, and small cell carcinomas.

## Fallopian Tube Theory of Ovarian Carcinogenesis

Serous tubal intraepithelial carcinomas are now known as the precursor lesions for high-grade serous carcinomas and spread from the open fimbriated ends of the fallopian tubes throughout the peritoneal cavity [9, 14]. Widespread use of the Sectioning and Extensively Examining the Fimbriated End (SEE-FIM) protocol by pathologists has improved detection of serous tubal intraepithelial carcinomas [15]. The idea that many ovarian cancers originate from these tubal lesions is supported by their co-existence in up to 60% of all high-grade serous ovarian carcinomas [16]. As this is 60%, at least 40% of high-grade serous cancers do not arise directly from the fimbriae.

## Risk and Protective Factors for Ovarian Cancer

**Genetic Risk Factors** Compared with women without known hereditary risk factors for ovarian cancer, women with deleterious genetic mutations are at markedly higher risk for ovarian cancer and are diagnosed at younger ages. Most hereditary ovarian cancers are linked to mutations in *BRCA1* or *BRCA2*, which confer a mean cumulative risk for ovarian cancer by age 70 of 40% and 18%, respectively [17, 18]. A recent international registry of 5689 women from 78 participating centers in 12 countries yielded similar estimates; the cumulative risk of ovarian cancer to age 80 was 49% for *BRCA1* and 21% for *BRCA2* mutation carriers [19]. Women with Lynch syndrome, though at relatively higher risk for colorectal and endometrial cancers, also have elevated risk of ovarian cancer. The cumulative risks for ovarian cancer by age 70 for the Lynch syndrome mutations in *MLH1* and *MSH2* are 11% and 15%, respectively, whereas no ovarian cancer-specific risk has been identified for mutations in *MSH6* or *PMS2* [20, 21].

Over the last decade, several moderate penetrance mutations have also been found to increase ovarian cancer risk. Mutations in *RAD51C* and *RAD51D* are estimated to increase risk for ovarian cancer by approximately 5-fold [22–24] and 12-fold [23–25], respectively. In *BRIP1* mutation carriers, the cumulative lifetime risk of developing ovarian cancer by age 80 is estimated at 5.8% [23, 18, 26].

**Protective Factors** Non-genetic factors that confer protection against ovarian cancer include pregnancy (risk decreases with each pregnancy) [27], breastfeeding (risk decreases with duration) [27], oral contraceptive pill use (risk decreases with duration) [28], and bilateral tubal ligation (risk decreases by ~ 50%) [29].

## Standard Approaches to Ovarian Cancer Screening

### Screening Trials for Women at Normal Risk of Ovarian Cancer

Eighty-five percent of ovarian cancers occur in women without increased hereditary risk. Since the outcome of screening for ovarian cancer requires a surgical procedure, a positive predictive value (PPV) of ≥ 10% is recommended to balance the benefits of screening against the harms of unnecessary procedures [30•]. Given the low prevalence of ovarian cancer (1/2500 postmenopausal women), a screening test for ovarian cancer requires a sensitivity for asymptomatic disease of > 75% and a specificity of > 99.6% to meet this criterion [31–33].

Efforts to detect ovarian cancer in early stage have utilized ultrasound imaging and blood tests, notably CA125. Transabdominal and, in more recent years, transvaginal sonography (TVS) have been evaluated in several large trials (see Table 1) with women at normal risk [34, 35]. CA125 (MUC 16) is a high molecular weight (5 MDa) heavily glycosylated transmembrane cell surface protein that is expressed by human epithelial ovarian cancers [42] and by normal

**Table 1** Ovarian cancer screening trials in normal-risk postmenopausal women

| Study Name Years | RCT | Location | # Women | Screening method | Aim | Outcome | Limitations/strengths |
|---|---|---|---|---|---|---|---|
| Kentucky study [34] | No | USA Kentucky | 37,293 screened | Annual TVS | 5-year OC survival: | Screened (74.8 ± 6.6%) vs unscreened (53.7 ± 2.3%) $P < 0.001$ Hard to interpret true effects of screening on disease mortality in non-RCT | Single-center Unscreened controls were diagnosed with OC in the center but not part of study Lead-time effect Healthy volunteer effect |
| SCSOCS[a] [35] 1985 to 1999 | Yes | Japan Shizuoka 212 hospitals | 82,487 total (41,688 screened) (40,799 control) | Annual CA125 (> 35 U/mL) Annual TVS | Early-stage I/II diagnosis: | Screened (63%) vs control (38%) $P = 0.23$ | |
| PLCO [36, 37] 1993 to 2001 | Yes | USA 10 sites | 78,216 total (34,253 screened) (34,304 usual care) | Annual CA125 (> 35 U/mL) (6 years) Annual TVS (first 4 years) | All-cause mortality: For invasive EOC, tubal and peritoneal cancer | 12.4-year risk ratio (1.18, 95% CI 0.91–1.54) 14.7-year risk ratio (1.01, 95% CI 0.97–1.05) | High surgical complication rate (15%) Lack of stage shift (22.2%) |
| UKCTOCS [38•] 2001 to 2005 | Yes | UK 13 sites | 202,638 total (50,639 USS) (50,640 MMS[b]) (101,359 control) | MMS[b] | Mortality reduction: For invasive EOC, tubal and peritoneal cancer Early-stage I/II diagnosis: | Primary analysis: Mortality reduction over years 0–14 was MMS 15% (95% CI − 3 to 30; $P = 0.10$) USS 11% (− 7 to 27; $P = 0.21$) Analysis excluding prevalent cases: Overall mortality reduction 20% (− 2 to 40) ($P = 0.021$) with reduction of 8% (− 27 to 43) over years 0–7 and 28% (− 3 to 49) mortality reduction over years 7–14 in favor of MMS Stage I/II shift with MMS of 36.1% stage I/II compared to control 23.9%, but not with USS ($P = .0001$) | Screening group had higher worry and lower pleasure scores when > 2 TVS performed as compared with no TVS [39] Good PPV of 4.4 surgeries per cancer detected [40] |
| NROSS [41] 2001 to 2011 | No | USA 7 sites | 4051 total | MMS[b] | Specificity and PPV of OC screening in PM women: | PPV of 40% (95% CI 12.2 to 73.8%) Specificity 99.9% (95% CI 99.7 to 100%) | |

[a] *SCSOCS* Shizuoka Cohort Study of Ovarian Cancer Screening

[b] MMS (ROCA CA125 + TVS) CA125 interpreted using a risk of ovarian cancer algorithm (ROCA) comparing each participant's CA125 value with its previous value, and testing for a change-point even at values < 35 U/mL. No change in CA125 led to annual CA125. Intermediate change in CA125 triggered a repeat CA125 in 3 months, and larger changes led to a secondary screen with TVS

endometrium, lung, and cornea. CA125 is cleaved and shed into body fluids where it can be measured by immunoassays [43, 44]. CA125 can be detected in blood from 90% of patients with stage III–IV and 50–60% of patients with stage I–II ovarian cancer [43].

When used alone on a single occasion, neither CA125 nor TVS have had adequate sensitivity nor specificity for early detection. The Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial was one of the first large trials of ovarian cancer screening for the general population. This study randomized 78,216 postmenopausal women ages 55 to 74 to receive either annual transvaginal ultrasound (TVS) with annual serum CA125 or conventional care [36]. Based on TVS or CA125 considered independently, multiple operations were performed for benign disease and there was no demonstrated mortality benefit of screening after a median follow-up of 14.7 years [37].

Greater specificity and sensitivity has been attained with two-stage strategies where rising CA125 has triggered TVS in 1–2% of participants. The UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) is the largest randomized clinical trial to evaluate ovarian cancer screening's impact on mortality in the general population [38•]. The trial randomized 202,638 postmenopausal women age > 50 to multimodal screening (MMS) (50,640), ultrasound screening (USS) (50,639), and 101,359 women to no screening between 2001 and 2005. The MMS arm analyzed the trend of annual CA125 values analyzed with a Bayesian risk of ovarian cancer algorithm (ROCA), comparing each participants current CA125 value with results of previous assays to detect a change-point that could occur within or outside the range of normal values < 35 U/mL [45]. Women whose CA125 did not change returned in a year. A marked increase in CA125 prompted TVS [39] and abnormal TVS surgical exploration. Intermediate change in CA125 leads to a repeat CA125 in 3 months. This approach attained a specificity of > 99.6% with only 4.4 operations per case of ovarian cancer detected [40]. The overall survival analysis was not statistically significant, but a mortality reduction was observed over the first 14 years for the MMS arm of 15% and USS of 11%. In a pre-specified subset analysis where prevalent cases were excluded, the overall average mortality reduction in incident cases with MMS vs no screening ($P = 0.021$) was 20% (− 2 to 40) with a reduction of 8% (− 27 to 43) in years 0–7 and 28% (− 3 to 49) in years 7–14, in favor of MMS. With wide confidence limits around these estimates, additional time will be required for the trial to mature and additional analysis will be performed. Sensitivity for early-stage disease was improved where 52.6% (70 of 133 women) were diagnosed using the ROCA with CA125 levels in the normal range (≤ 35 U/mL), and 41.4% (55 of 133 women) were diagnosed in early stages I/II [40], reflecting a stage shift.

Similar specificity has been observed in the Normal Risk of Ovarian Cancer screening study (NROSS) of 4051 postmenopausal women in the USA who had yearly CA125 values interpreted using the ROCA using a protocol identical to the MMS arm of the UKCTOCS. Over the last 18 years, in parallel with the UKCTOCS, 21 women have undergone surgery based on the ROCA algorithm. Thirteen have had epithelial ovarian malignancies with 11 invasive cancers and 2 borderline tumors including 9 cases in stage I/II. Like the UKCTOCS, specificity and PPV were high with 3 procedures for each case of ovarian cancer detected [41].

With an update of the UKCTOCS still pending, the US preventative services task force (USPSTF) concluded that screening for ovarian cancer for normal-risk women does not reduce mortality and is not recommended [46].

## Screening Trials for Women with Elevated Risk of Ovarian Cancer

The National Comprehensive Cancer Network (NCCN) clinical practice guidelines recommend risk-reducing salpingo-oophorectomy (RRSO) upon completion of childbearing or between 35 and 40 years for *BRCA1* and between 40 and 45 years for *BRCA2* mutations carriers. Despite the proven benefit of RRSO for ovarian cancer risk reduction and mortality [47], uptake of RRSO is variable among high-risk women. Many complex factors influence the decision to undergo RRSO, in these premenopausal women including fear of menopause, fear of cancer, family history of cancer, and perceived risks and benefits of surgery [48]. Despite uncertain benefits, current NCCN guidelines allow for screening with TVS combined with CA125, starting at 30–35 years, as an alternative for high-risk women who have not yet undergone RRSO [].

The UK Familial Ovarian Cancer Screening Study (UKFOCSS) enrolled 4348 women with an elevated risk of EOC or fallopian tube cancer with a median follow-up time of 4.8 years. Women underwent ROCA screening every 4 months with annual TVS if normal. If ROCA values were elevated, TVS was recommended within 2 months. After a median follow-up time of 4.8 years, 19 cases of EOC or fallopian tube cancer were diagnosed, with ten (52.6%) at stage I/II, indicating a stage shift [49•]. Whether these encouraging early findings will translate into lower mortality is not yet known, as data collection is ongoing.

Skates and colleagues reported in 2017 on the early detection of ovarian cancer using ROCA screening with every 3 months of CA125 testing in 3692 high-risk women by combining data from the Gynecologic Oncology Group (GOG) [50] and Cancer Genetics Network (CGN) trials. Similar to the UKFOCSS trial, about half of incident cancers were diagnosed at an early stage, and ROCA detected 3/6 (50%) before

CA125 exceeded 35 U/mL. Screening yielded a relatively high specificity of 92% [51•].

## Novel Approaches to Early Detection

In patients at average and elevated risk of ovarian cancer, two-stage approaches to early detection appear promising where rising levels of blood biomarkers over time prompt imaging in a small fraction of patients, enhancing specificity and reducing stress, cost, and possible morbidity. Clearly, however, there is an unmet need for greater sensitivity in both stages of screening.

### Protein Biomarkers

Over the last 3 decades, more than 100 blood biomarkers have been evaluated for their ability to detect early-stage ovarian cancer, individually and in combination with CA25. Human epididymis protein 4 (HE4) has two whey acidic protein (WAP) domains and a 4-disulfide core (WFDC2) and is secreted by epithelial ovarian cancer cells. HE4 is increased in at least 70% of ovarian cancers, but unlike CA125 is less frequently elevated by benign disease and can detect a fraction of cases with normal CA125 [52]. CA72.4 is an antigenic determinant on a 200–400 KD glycoprotein that can detect cases of ovarian cancer missed by CA125 and HE4.

Moore et al. showed that when combined, HE4 and CA125 produced the highest sensitivity at 76.4% (specificity 95%) of other combinations of markers and increased the sensitivity of CA125 alone for differentiating benign verses cancer in pelvic masses [53]. Cramer et al. tested a panel of biomarkers on serum samples from the PLCO study. The top markers were CA125 (86%), HE4 (73%), transthyretin, CA72.4, and CA15.3, with sensitivities declining > 6 months from diagnosis [54]. Four biomarkers including CA125, HE4, CA72.4, and CA15.3 were tested in 810 invasive EOCs cases and controls from the European Prospective Investigation into Cancer and Nutrition (EPIC) study. Receiver operator performance was highest for CA125 (0.92), followed by HE4 (0.84), CA72.4 (0.77), and CA15.3 (0.73), with lower performance in earlier staged disease and with increasing time between sampling and diagnosis [55]. Using serum samples from the UKCTOCS trial, HE4 and CA72.4 detected 16% of cases missed by CA125, but did not provide additional lead time [56].

### Autoantibodies

The limited ability of protein biomarkers to detect early ovarian cancer may be due to the small size of early cancers and/or to the low expression or shedding of biomarkers. Autoantibodies could, however, be stimulated by small volumes of cancer [57]. The tumor suppressor gene *TP53* is mutated in almost all high-grade serous ovarian cancers [58]. TP53 autoantibody levels are increased in more than 20% of pre-treatment sera from patients with both early- and late-stage ovarian cancer. Notably, TP53 autoantibody levels were elevated in 16% of patients whose cancer was not detected with ROCA-interpreted CA125 during the UKCTOCS trial. TP53 autoantibody levels rose 8 months prior to CA125 when it was elevated and 22 months prior to diagnosis when CA125 did not rise [59•].

A recent systematic review of the world literature by Fortner [60] identified additional evidence for the TP53 autoantibody as well as several other candidate autoantibodies for early detection of ovarian cancer that were elevated in a significant fraction of cancers, including anti-homeobox gene A7 (HOXA7) [60] and anti-interleukin 8 (IL-8) [61]. Panels of autoantibodies are currently being evaluated by the National Cancer Institute Early Detection Research Network to complement CA125 and to enhance the sensitivity of two-stage strategies where rising blood biomarkers prompt TVS.

### MicroRNAs

MicroRNAs (miRNAs) circulate in many body fluids as stable entities bound to proteins (e.g., Argonaute 2) or packaged in exosomes, microvesicles, or apoptotic bodies [62]. miRNAs are single stranded, short (~ 22 nucleotide), non-coding RNA that regulate post-transcriptional gene expression, typically leading to translational repression [62, 63]. Since circulating miRNAs change based on pathologic states, they are considered potential cancer biomarkers [62, 64], and several miRNA profiles show significant promise as EOC biomarkers. Four serum miRNAs (miR-182, miR-200a, MiR-200b, miR-200c) are elevated in blood from serous EOC patients, and miR-200b combined with miR-200c performed well in distinguishing serous EOC from controls (AUC = 0.784) [65]. The combination of miR-205 and let-7f showed excellent accuracy for EOC (AUC = 0.831 (95% CI 0.772–0.880) with high sensitivity (62.4%) and specificity (92.9%), especially in patients with stage I disease [66]. An eight-miRNA panel also detected early-stage EOC from benign tumors with 86% sensitivity and 83% specificity [67]. Combining small RNA sequencing from 179 human serum samples with a neural network, Elias and colleagues produced an miRNA algorithm for the diagnosis of EOC with the most accurate performance to date (AUC 0.90; 95% CI 0.81–0.99) [68•]. Although miRNA has significant potential as a biomarker, comparison and combination studies with CA125, HE4, and TVS in a prospective trial are lacking.

## Circulating Tumor DNA

Circulating tumor DNA (ctDNA) is the fraction of cell-free DNA that contains tumor-specific somatic mutations that are found in the circulation [69]. Analysis of serum ctDNA ("liquid biopsy" [70]) provides a potential noninvasive method of tumor detection and monitoring [70, 71•] and is especially advantageous for cancers that are difficult to biopsy. In a small sample of 7 patients with ovarian cancer, Bettegowda et al. found 100% had detectable ctDNA [71•]. ctDNA has also been identified following uterine lavage in 24 (80%) of 30 patients with ovarian cancer and 5 of 5 with endometrial cancers; however, mutations were also found in 8 (27%) of 27 patients with benign lesions [72]. Detection of ctDNA has improved with targeted deep sequencing, which in one study identified mutations at allelic frequencies of 2% with > 97% sensitivity and specificity.

Cohen and colleagues created CancerSEEK, which concurrently evaluates 8 protein biomarkers, including CA125, and the presence of mutations in 1933 genomic positions including single base substitutions, insertions, or deletions [73•]. In a case-control study that included eight cancer types, CancerSEEK was most accurate in ovarian cancer, with a sensitivity of 98% and specificity of > 99% with only 7 positives out of 812 patients without known cancers. However, a majority of patients with ovarian cancer had stage III disease where CA125, one component of CancerSEEK, would detect 90% of cases. Some of the drawbacks to ctDNA include detection of mutations in non-cancer patients and poor early-stage detection.

## DNA Methylation

DNA promoter hypermethylation of tumor suppressor genes is now known to occur in most cancers, resulting in the inactivation of tumor suppressor genes [74]. Promoter methylation usually occurs early during carcinogenesis, and is therefore a potential early tumor marker [74, 75]. A panel of 3 DNA methylation serum markers was tested in 250 EOC patients undergoing chemotherapy, and a large cohort of patients from the UKCTOCS trial. Serum DNA methylation markers detected over half of the ovarian cancer cases in serum samples acquired up to 2 years prior to diagnosis [76]. Prospective studies are still needed [75, 77].

## Imaging

TVS is the gold standard imaging modality for viewing the adnexa and uterus. In the PLCO trial, TVS had a PPV of 1% and CA125 (> 35 U/mL) had a PPV of 2.6%. A combination of abnormal TVS and elevated CA125 increased the PPV to 23.5%, but 60% of ovarian cancers would have been missed. The main drawbacks to TVS are the inability to visualize small lesions on the ovaries and the failure to visualize the fimbriae of the fallopian tubes [78•]. In the UKCTOCS, the ROCA detected an early rise in the CA125 level in a significant number of screened subjects destined to develop ovarian cancer where the TVS was normal, resulting in a delay in surgery. Early-stage ovarian lesions are now estimated to be < 3 mm and may persist at this size for several years, requiring imaging techniques that can detect very small tumors [79, 80].

Both magnetic resonance imaging (MRI) and magnetic relaxometry (MRX) can detect and locate magnetic nanoparticles without ionizing radiation [81]. Superconducting quantum interference device (SQUID) technology can detect delays in magnetic relaxation when nanoparticles conjugated to a monoclonal antibody reactive with ovarian cancer are bound to cancer cells, differentiating them from unbound normal cells [81]. This technique has been used in several tissue models including breast cancer and leukemia and holds potential for the detection of early ovarian cancer [81, 82]. Williams et al. engineered an optical sensor made up of an HE4 antibody-carbon nanotube complex which measures HE4 in different body fluids. The sensors were then implanted into four mouse models of ovarian cancer and were capable of noninvasive cancer biomarker detection [83].

Doppler ultrasound is currently used to detect central ovarian blood flow when differentiating benign from ovarian malignancies, but it is unable to detect small ovarian cancers [78•, 84]. The use of microbubble contrast agents with ultrasound has allowed visualization of neovascularity in some ovarian masses [85]. In a study by Xiang et al., 3-D microbubble contrast-enhanced TVS differentiated benign from malignant small ovarian masses with high sensitivity (100%) and specificity (98%) [78•, 86]. However, this method does not overcome the challenge of imaging the fallopian tubes or detecting small ovarian lesions.

Light-induced endogenous fluorescence (autofluorescence) has been used to detect precancerous lesions in other organ systems, including the cervix. Autofluorescent imaging of surgically removed fallopian tubes had a sensitivity of 73%, a specificity of 83%, and a PPV of 57% for ovarian or serous tubal intraepithelial carcinomas. A next step in the development of this technology will be in vivo screening via falloposcopy [87].

## Fallopian Tube Cytology and Tumor DNA Detection in Pap Smears

Recognition that many ovarian cancers arise in the fallopian tube has stimulated development of tissue sampling methods specifically for the fallopian tube. Although reproductive endocrinologists once used falloposcopy to visualize the interior of the fallopian tubes, this practice has been mostly abandoned [88]. More recently, in a small feasibility study, Lum et al. attempted cytologic sampling of the fallopian tubes using

hysteroscopic brush cytology without visualization. Falloposcopy was attempted and while unsuccessful for the hysteroscopic portion, was successful for the laparoscopic assessment [89]. A recent study proposal by Gizzo et al. will utilize a 4.9-mm-integrated in-office hysteroscope to cannulate the fallopian tube using a 4 French (1.3 mm) sterile ureteric drainage catheter, with the goal of reaching the fimbriated ends. Following instillation of normal saline, the fluid containing the end-luminal fallopian cells will be aspirated and analyzed with cytology [90]. Several groups have started to characterize the cytology of the fallopian tube as well as endometrial cytology for early detection of ovarian cancer, although further analysis and standardization is needed [91–94].

Liquid-based cervical Pap smear testing, which includes both cytological analysis and DNA detection, has dramatically reduced cervical cancer mortality and has recently been studied as a screening tool for other cancers. Kinde et al. identified mutations from DNA in liquid Pap smear specimens in 100% of endometrial cancers and 41% of ovarian cancers [95]. Another approach is PapSEEK, which detected endometrial and ovarian cancers based on DNA analysis from routine Pap tests by the detection of 18 genes and aneuploidy. The sensitivity for ovarian cancer was 63%, with a specificity of ~ 100%, but required the use of an endometrial brush rather than routine Pap brush [96]. Analyzing ctDNA from the cervix and blood has detected 55% of early-stage ovarian cancers [97].

## Challenges and Future Directions

Despite extensive investigation, a single marker for early-stage ovarian cancer remains elusive, and there is no currently accepted method for ovarian cancer screening. Examination of CA125 over time as opposed to a single time point still demonstrates the best sensitivity and specificity as a marker, although its value for future screening purposes may require complementary markers. Ongoing data collection will help determine whether earlier detection of ovarian cancer through multimodal screening yields reductions in mortality over the long term, as suggested by late trends in the UKCTOCS study. The standard of care worldwide is RRSO by age 40 for BRCA1 and 45 for BRCA2. Until high-risk women undergo surgery, an affective screening strategy would be helpful. The low incidence of ovarian cancer remains a challenge for identifying effective early detection strategies. Specificity needs to be > 99.6% in order to avoid harm in healthy women. Imaging needs to be able to visualize early-stage I/II lesions yet remain cost effective. The number of women required for a prospective trial with the gold standard of mortality reduction as an end-point is also a challenge.

In the meantime, the treatment of women at high genetic risk of ovarian cancer is highly variable across the world. Many countries recommend RRSO, particularly within Europe and the UK, without screening options [98]. Within the US, RRSO is also recommended, but the NCCN includes screening with CA125 (> 35 U/mL) and TVS as an option prior to RRSO.

Several protein biomarkers show potential to enhance screening and early detection. The addition of HE4 and CA 72.4 to CA125 detected 16% more than CA125 alone. The TP53 autoantibody was able to detect 16% of EOC patients that CA125 missed, indicating a possible use as an adjunct to conventional CA125 screening. A panel of autoantibodies might be even more effective in complementing CA125. The possibility of a "liquid biopsy" is within closer reach as detection of circulating tumor DNA has improved over time. Other tests based on DNA methylation assays, microRNA algorithms, and Pap-like cytologic analysis show potential. Of these, PapSEEK holds the greatest potential for reaching the largest number of patients; however, its value hinges on a validation study, cost, and performance with the liquid Pap, since available worldwide. The UKCTOCS has a serum bank that may allow validation of these newer markers.

## Conclusion

There remains intense interest from patients and the medical community to identify an effective strategy for the detection of early ovarian cancer. There are a number of promising strategies focusing on new biomarkers and new imaging techniques. Current two-stage multimodal ovarian cancer screening algorithms incorporating ROCA-interpreted CA125 with TVS have shown a stage shift to earlier (stage I/II) cancers in high-risk women and may ultimately demonstrate long-term effects on mortality. In the absence of a superior replacement, it is reasonable to offer two-stage screening to high-risk women who have not yet undergone RRSO. The addition of imaging to detect small fallopian tube or peritoneal lesions along with the further development of molecular techniques holds promise for supplementing or surpassing the two-stage screening test.

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## Compliance with Ethical Standards

**Conflict of Interest** Denise R. Nebgen declares that she has no conflict of interest.

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Papers of particular interest, published recently, have been highlighted as:
- • Of importance
- •• Of major importance


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