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Early Detection of Ovarian Cancer

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1. Introduction

The rationale for early detection of ovarian cancer is compelling. Ovarian cancer confined to the ovaries (stage I) can be cured in up to 90% of patients, and disease confined to the pelvis (Stage II) is associated with a 5-year survival of 70%. However, disease that has spread beyond the pelvis (stage III-IV) has a long-term survival rate of 20% or less. Only 20% of ovarian cancers are currently diagnosed in stage I-II [1]. Computer simulations suggest that detection of preclinical disease at an earlier stage could improve survival by 10–30% and would be cost-effective [2,3].

The clinical requirements for early detection are stringent. Given the postmenopausal prevalence of 1:2,500, effective screening requires not only high sensitivity for pre-clinical disease of ≥ 75%, but also very high specificity of ≥ 99.7% to achieve a positive predictive value (PPV) (Table 1) of 10% (i.e., 10 operations for each case of ovarian cancer detected). Increasing specificity, rather than improving sensitivity alone or screening only high-risk patient subsets, will have the greatest impact on the positive likelihood ratio (LR+) of a test result (Table 2).

Successful early detection strategies for ovarian cancer should diagnose more high grade epithelial ovarian cancers at an early stage and improve outcomes, i.e. overall survival [4]. However, this relies on two basic assumptions [5]. First, one assumes that high grade epithelial ovarian cancers currently diagnosed at an advanced stage, if detected earlier, will have the same favorable prognosis as Stage I cancers, which are heterogeneous and include low grade

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neoplasms and non-serous histotypes. Secondly, determinations of screening efficacy must control for lead-time bias. Any earlier diagnosis, irrespective of whether it impacts overall survival, will add to a patient's total survival time from diagnosis. While the survival proportion at any given time point is increased, there is no true improvement in the patient's life expectancy [6]. Several trials have examined different screening strategies, most notably the Prostate, Lung, Colorectal and Ovarian (PLCO) Screening Trial, the Normal Risk Ovarian Screening Study (NROSS), and the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS).

Transvaginal sonography (TVS) and the protein biomarker CA125 are the two best studied screening tools for ovarian cancer. However, neither is sufficient for general screening, exemplified by PLCO Screening Trial [7]. PLCO enrolled more than 70,000 post-menopausal women between 1993 and 2001 to receive usual care or in 39,110 to undergo annual screening with CA125 and TVS, or, in the later rounds of the study, CA125 alone. Overall, 388 ovarian cancers were diagnosed, but 1080 women underwent surgery for a false positive result, with 15% experiencing major complications. Moreover, screening failed to improve survival. With a median follow-up of 14.7 years in each arm, the ovarian cancer death risk ratio was 1.06 (95% CI: 0.87–1.30) between the two groups [8].

The failure of PLCO highlighted the need for a two-tiered screening strategy. Ovarian cancer is associated with rising CA125, and benign disease is not [9]. The Risk of Ovarian Cancer Algorithm (ROCA) relies on each woman's own baseline CA125 value to determine whether there has been a significant increase [10]. Over the last 17 years in NROSS, 34,637 samples have been obtained from 5,729 postmenopausal women at conventional risk [11]. Rising CA125 judged by the ROCA prompted TVS, and sonographic findings compatible with cancer led to exploratory surgery. Less than 0.9% of these women have been referred for ultrasound after each annual screening, and 2.6% over multiple years on study.

In UKCTOCS >200,000 postmenopausal women at average risk were randomized to three groups: control (101,359), annual TVS (50,639) and annual CA125 with ROCA prompting TVS (50,640) [12]. With the algorithm, only 3–4 operations were required to detect each case of ovarian cancer. Excluding prevalent cases and primary peritoneal disease, a 20% reduction in mortality was observed (P=0.021). The statistical bounds around the estimate of reduction in mortality were, however, large and additional follow-up will be required to validate this estimate.

In the absence of definitive data, early detection of ovarian cancer remains a critical unmet public health need. In 2018, updated recommendations from the US Preventive Services Task Force (USPSTF) conclude with "moderate certainty" that "the net balance of the benefit and harms of screening is negative" and recommend against screening in average risk asymptomatic women [13]. For women at increased genetic risk (i.e., BRCA1/2 mutation carriers) who delay prophylactic bilateral salpingo-oophorectomy, screening with semi-annual CA125 and TVS is recommended, but there is not yet definitive evidence that this strategy detects fallopian or ovarian cancer earlier or improves outcomes [14]. Two ongoing clinical trials will hopefully shed light on whether use of the ROCA algorithm for screening triage will impact mortality in this population [15,16]. Regardless, there remain opportunities

to improve the two-tiered screening approach. The discussion below focuses on additional screening strategies beyond CA125 and TVS.

2.1 Protein biomarkers.

CA125 remains the most sensitive and specific protein biomarker for detecting early stage disease in apparently healthy populations. CA125 is a high molecular weight (~5 MDa) heavily glycosylated membrane-spanning mucin (MUC16) glycoprotein. The extracellular domain of MUC16 is cleaved near the ovarian cancer cell surface, releasing CA125 into the peri-cellular space and ultimately into the blood where it can be measured with an immunoassay. CA125 levels are elevated in blood from >90% of patients with advanced stage (III-IV) and in 50–60% with stage I ovarian cancer [17].

More than 110 potential protein biomarkers have been evaluated individually and in combination with CA125 [18]. Other top candidates include HE4, transthyretin, CA15.3, and CA72.4 [19]. HE4 (human epididymal protein 4) is a 124-amino acid glycosylated whey protein that is elevated in sera from approximately 60–75% of ovarian cancer patients and that detects a small fraction of cases missed by CA125. CA15.3 and CA72.4 are distinct epitopes on the MUC1 mucin.

Terry, *et al*, measured CA125, HE4, CA72.4, and CA15.3 in 810 invasive epithelial ovarian cancer cases and 1,939 controls from Phase III specimens from the European Prospective Investigation into Cancer and Nutrition study [20]. All the markers performed best within 6 months of diagnosis, but the capacity to discriminate between future case patients and non-cases dropped rapidly with increasing time from blood collection to clinical diagnosis. Successive additions of CA125, HE4, CA72.4 and CA15.3 as pre-diagnostic predictors of future ovarian cancer diagnosis could improve the model C-statistic, but only minimally compared to a model based on CA125 alone (0.70 to 0.71). Our own studies indicate that the addition of HE4 and CA72.4 detects 18% of cases missed by CA125, but does not provide diagnostic lead time in specimens from the UKCTOCS trial [18].

2.2 Autoantibodies

Autoantibodies to mutant proteins can be stimulated by small volumes of cancer in the ovary or fallopian tube, providing greater sensitivity and earlier detection than CA125 or other assays that detect shed biomarkers. Autologous antibodies can be produced against mutant TP53 protein. Alteration in *TP53* is the most common genetic mutation among ovarian cancers, seen in up to 96% of high-grade serous carcinomas [21]. At a specificity of 97%, autoantibodies could be detected in 21–30% of serum samples from ovarian cancer patients from MD Anderson, the Australian Ovarian Cancer Study and the UKCTOCS [22]. Among 164 cases with rising CA125 detected in serial preclinical serum samples with the ROCA, 20.7% had elevated TP53 autoantibody. Of the 34 ovarian cancer cases detected with the ROCA, TP53 autoantibody titers were elevated 8 months before CA125. In the 9 cases missed by the ROCA, TP53 autoantibody was elevated 22.9 months before cancer diagnosis. Consequently, TP53 autoantibody levels provide the first bio-marker with clinically significant lead time over elevation of CA125 or an elevated ROCA value.

Kaaks, *et al*, performed a prospective analysis on a selected panel of four autoantibodies—against TP53, CTAG1A, CTAG2 and NUDT11— using serum samples collected up to 36 months before diagnosis from 194 ovarian cancer patients and 705 matched control participants [23]. With lead times less than or equal to 6 months, sensitivity for early detection ranged from 19–23% for the four autoantibodies at 98% specificity, but with lead time of greater than 1-year, sensitivity ranged from only 1–11%. Addition of the four autoantibodies to CA125 did not improve sensitivity for detection at 98% specificity, although serial preclinical specimens were not analyzed. A recent review of the world literature has reported that 6 individual autoantibodies against EpCAM, IL-8, PLAT, MDM2, c-Myc and HOXA7 provide 39–67% sensitivity at 98–100% specificity for detecting ovarian cancer at all stages [24]. These and other candidates are being evaluated in combination with protein biomarkers.

2.3 Circulating tumor DNA

Circulating cell-free DNA (cfDNA) in serum and plasma can be distinguished from lymphocyte DNA by size; circulating DNA is fragmented to an average length of 140 to 170 base pairs (bp) [25]. Efforts have focused on the fraction of circulating DNA derived from tumors, called circulating tumor DNA (ctDNA) [26]. ctDNA is released from tumor cells primarily through apoptosis [27,28]. The ability to perform deep sequencing and droplet digital PCR (ddPCR) on minute quantities of ctDNA has led to the ability to detect specific mutations, loss of heterozygosity (LOH), DNA hypermethylation, copy number variation, and even the presence of single nucleotide variants [29–33]. Swisher, *et al*, used traditional PCR to identify *TP53* mutations in cfDNA. Of the 69 cases with somatic *TP53* mutations, tumor-specific *TP53* sequences were detected in 21 (30%) plasma or serum samples [34]. However, mutant *TP53* was detected in only one case of Stage I cancer.

Detection of ctDNA has improved with the development of technologies with deeper sequencing coverage. Whereas targeted sequencing for ctDNA can detect mutations with an allelic frequency down to 5%, tagged amplicon sequencing (TAm-Seq), which uses a combination of short amplicons, two-step amplification, sample barcodes, and high-throughput PCR, can identify allelic fractions as low as 2% [35]. The assay has been evaluated in plasma from patients with advanced stage high-grade serous ovarian cancer and shown to have 97% sensitivity and specificity [35]. Further refinements of this technique have been reported to detect allelic fractions down to 0.02% [36]. How these more advanced detection methods will translate to early stage cancers is unclear. In fact, these improvements in sensitivity may come at a specificity cost. Using duplex sequencing, Krimmel, *et al*, was able to detect extremely low frequency *TP53* mutations (median mutant fraction 1/13,139) in peritoneal fluid, but the authors found mutations in nearly all study subjects, whether with or without cancer (35/37) [37]. This speaks to the occurrence of low level mutant TP53 events in normal physiology [38].

As a high sensitivity test, ctDNA may have a role in complementing CA125. In a multi-cancer combined ctDNA and protein biomarker panel called CancerSEEK, 46/54 (85%) of the ovarian cancers were identified largely by *TP53* mutations and CA125 [39]. While the overall panel had 98% reported sensitivity for ovarian cancer, most were advanced stage high grade serous tumors, with only 9 cases of Stage I disease.

2.4 DNA methylation

Hypermethylation of tumor suppressor promoters and hypomethylation of oncogenes are frequent genetic events [40–42]. Methylation-specific PCR (MSP) is very sensitive, able to identify 1 methylated allele in 1000 unmethylated alleles [43]. The frequency of promoter hypermethylation increases with advancing disease stage [44]. Using multiplexed MSP to examine cfDNA for seven candidate genes (*APC*, *RASSF1A*, *CHDH1*, *RUNX3*, *TFP12*, *SFRP5*, and *OPCML*), Zhang, *et al*, reported 85% sensitivity at 91% specificity for early stage ovarian cancer compared to a single CA125 value, which produced a sensitivity of 56% at 64% specificity. However, this was based on only 17 early-stage patients [45]. More recently, Widshwendter¸*et al*, described a three-DNA-methylation-serum-marker panel developed from 699 cancerous and non-cancerous tissue samples [46]. They used targeted ultra-high coverage bisulfite sequencing in 151 women and validated in 250 women with various conditions, including those associated with high CA125 levels (endometriosis and other benign pelvic masses), serial samples from 25 patients undergoing neoadjuvant chemotherapy, and a nested case control study of 172 UKCTOCS control arm participants. The marker panel discriminated high grade serous ovarian cancer patients from healthy women or patients with a benign pelvic mass with 41.4% sensitivity at 90.7% specificity. When applied to serum samples collected 1–2 years before an ovarian cancer diagnosis, the methylation panel had 16.7% sensitivity at 96.9% specificity.

2.5 Circulating miRNA

miRNAs are short (18–24 nucleotide) non-coding RNAs that regulate gene expression through post-transcriptional modification of mRNA transcripts [47]. An individual miRNA may regulate several different genes within a pathway; thus, knowing information about a relatively small number of miRNAs can convey information about thousands of target genes [48]. miRNAs can circulate either bound to the chaperone protein Argonaute 2 (Ago2) or contained within extracellular vesicles (EVs) [49]. They are highly stable in circulation and resistant to ribonucleases [50]. An important property of miRNAs is that they act in a coordinated fashion. Thus, any single miRNA is unlikely to be a reliable biomarker, as compared to a miRNA panel. Using 8 miRNAs, Yokoi *et al* were able to distinguish early stage ovarian cancers from benign tumors with 86% sensitivity and 83% specificity. Additionally, miRNAs were detectable in EVs collected from cultured ovarian cancer cell lines [51].

Our group has shown that the specificity of miRNA prediction models can be improved by combining next generation sequencing technology with machine learning algorithms [52]. A neural network prediction model was derived using serum miRNA-seq from 98 incident cases of invasive ovarian cancer, including 53 cases of Stage I or II disease, and applied to an independent 454-patient sample set with a disease prevalence of 3.3%. At a sensitivity of 75% and specificity of 100%, the model had an AUC of 0.92 (95% CI 0.82–1.00). Among samples where CA-125 data were available, neither the miRNA signature nor any individual miRNA correlated with CA-125 levels, suggesting miRNAs as an independent disease marker.

2.6 Proximate tumor fluids

Because there is continuity between the distal fallopian tube and the vagina, the use of body fluids more proximate to the ovary as screening tools is of interest. Somatic mutations in *TP53* have been isolated from tampons of women with ovarian cancer [53]. This has also been achieved through uterine lavage [54]. Using multiplexed PCR to detect 18 mutations or aneuploidy in endocervical brushings from 656 patients with endometrial or ovarian cancers and 1002 healthy controls, Wang, *et al*, showed that their test, called PapSEEK, had 33% sensitivity at 99% specificity for ovarian cancer. This improved to 45% sensitivity and 100% specificity in a smaller cohort of 299 women assessed with an intrauterine brushing [55].

3.0 Novel imaging techniques

TVS is the preferred clinical modality for imaging the adnexa. TVS can be delivered at most centers, at low cost, without radiation, and with minimal discomfort to patients [56]. Among women with an adnexal mass, morphology indexing has a high NPV of 0.997 for excluding malignancy [57]. Doppler flow studies have improved the specificity of TVS in experienced hands. However, one drawback of TVS is that the resolution of sonographic imaging is insufficient to diagnose very small invasive or pre-invasive lesions. Failure to image fallopian tubes is a particularly important limitation in that many high grade serous ovarian cancers are believed to arise from epithelial cells on the fimbriae of the fallopian tubes [38].

Microbubble contrast holds promise and has improved the ability to distinguish benign from malignant adnexal masses but is not likely to improve detection of fallopian tube lesions. Hyperpolarized ^{13}C MRI has shown a unique signature in prostate cancers and might prove useful in ovarian cancer [58]. Magnetic relaxometry (MRX) is another modality that might substantially enhance sensitivity by two orders of magnitude [59]. Superconducting Quantum Interference Detection (SQUID) can measure delays in magnetic relaxation of antibody-coated iron oxide nanoparticles. Such delays are observed when nanoparticles bind to cancer cells, but not when they are free in the blood or peritoneal cavity. This modality has been applied to detecting breast cancer cells in murine xenografts, minimal residual disease in leukemic bone marrow biopsies, and measuring nanoparticle accumulation in biological samples [60,61]. Studies are currently being conducted with human ovarian cancer xenografts, but clinical studies have not yet been performed.

4. Conclusion

Early detection of ovarian cancer remains an important but, to date, an elusive goal. Efforts to develop efficient and cost-effective ovarian cancer screening have been hampered by the low prevalence of this cancer. A common theme from the clinical trials testing various screening methods is that no single marker has the test characteristics necessary to be a standalone screening test, and to date, no effective strategy exists. Rather, multimodal assessments based on dynamic and algorithmic models are more likely to produce the specificity required for clinical development. Two stage strategies where rising values for blood tests trigger imaging have attained adequate specificity, but not at acceptable sensitivity. Circulating protein biomarkers, autoantibodies, ctDNA and miRNA and

proximate fluid collection all deserve further evaluation to enhance the sensitivity of the initial screening stage. In the end, cost effective screening is likely to depend upon an extremely high specificity first screen, followed by a more sensitive secondary imaging strategy.

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SYNOPSIS:

Early detection of ovarian cancer could reduce mortality by 10–30%. Given the low prevalence of ovarian cancer in postmenopausal women (1:2500), effective screening requires high sensitivity (>75%) and extremely high specificity (99.7%). Clinical trials suggest the best specificity is achieved with two-stage strategies where rising serum CA125 triggers transvaginal sonography to detect a malignant pelvic mass, although any evidence for such approaches improving overall survival has been limited. Screening may be improved by combining CA125 with novel biomarkers, such as autoantibodies, circulating tumor DNA (ctDNA) or microRNAs. In order to detect pre-metastatic ovarian cancers originating in the distal fallopian tube, more sensitive approaches to diagnostic imaging will be required.

KEY POINTS

- Given the low prevalence of ovarian cancer even among postmenopausal women (1:2500), an effective screening strategy requires high sensitivity (>75%) and extremely high specificity (99.7%).
- Screening trials in the United States and the United Kingdom indicate sufficient specificity using a two-stage strategy of rising CA-125 levels with subsequent triage to transvaginal ultrasound.
- Additional protein biomarkers may provide only a modest improvement upon CA125 alone, but there is increasing evidence for the potential for autoantibodies, ctDNA, and microRNAs in the blood or fluid from the fallopian tube, uterus or cervix to complement CA-125.
- More sensitive imaging will be required to detect early stage lesions in the ovary and particularly in the fallopian tube.

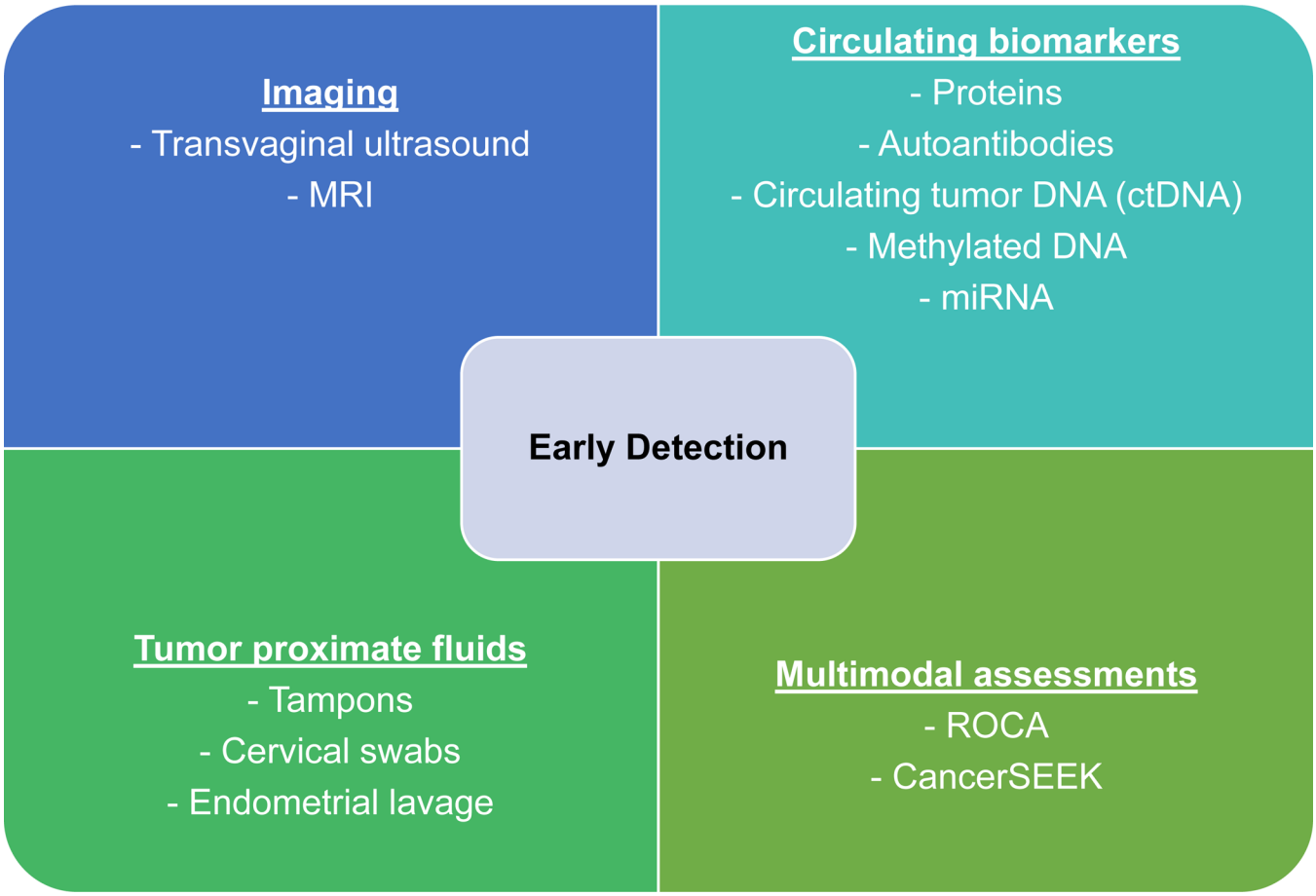

Figure 1.
Potential Biomarkers for early detection

Table 1.

Key statistical terminology in the context of population screening tests for ovarian cancer

Characteristic	Synonyms	Definition
Prevalence	Pre-test probability	Proportion of population affected by a condition
Sensitivity	Detection rate; True positive rate (TPR)	Proportion of subjects with cancer who test positive
Specificity	True negative rate (TNR)	Proportion of subjects without cancer who test negative
False positive rate (FPR)	1 - specificity; Type I Error; α	Proportion of subjects without cancer who test positive
False negative rate (FNR)	1 - sensitivity, Type II error; β	Proportion of subjects with cancer who test negative
Positive Predictive Value (PPV)	Positive post-test probability	Probability that a subject with a positive test has cancer
Negative Predictive Value (NPV)	Negative post-test probability	Probability that a subject with a negative test does not have cancer
Positive likelihood ratio (LR +)	TPR / FPR	Ratio between the probability of a positive test result given the *presence* of the disease and the probability of a positive test result given the *absence* of the disease
Negative likelihood ratio (LR-)	1 – TPR / specificity	Ratio between the probability of a negative test result given the *presence* of the disease and the probability of a negative test result given the *absence* of the disease
Odds Ratio	[TPR / (1 − TPR)] × [(1 − FPR) / FPR]	
Receiver-Operating Characteristic (ROC) Curve		Plot of sensitivity vs. FPR
Area under the ROC Curve (AUC)	C-statistic	A normalized Mann Whitney/Wilcoxon test where the Wilcoxon statistic is divided by the product of the number of individuals in the two groups measured
Accuracy		Overall probability that a subject will be correctly classified

Table 2.

Performance of a model ovarian cancer screening test based on changing test characteristics

	Model test				
	Baseline test characteristics	**Higher sensitivity**	**Higher specificity**	**Testing higher risk population**	**Ultra-high specificity**
Prevalence	1:2500	1:2500	1:2500	1:250	1:2500
Sensitivity	75%	95%	75%	75%	75%
Specificity	98.0%	98%	99.73%	98%	99.98%
PPV	1.48%	1.90%	10.0%	13.04%	60%
NPV	99.99%	99.998%	99.99%	99.90%	99.99%
Accuracy	97.99%	98.04%	99.72%	97.90%	99.97%
LR+	37.5	48.45	277.67	37.35	3750
LR−	0.26	0.05	0.25	0.26	0.25