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Review

Screening for Ovarian Cancer in the General Population: State of Art and Perspectives of Clinical Research

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Abstract. *Background/Aim: Screening for ovarian cancer in the general population is a challenging issue. The aim of this review was to analyze both the studies based on serum CA125 assay and ultrasound (US) and the novel perspectives of clinical and biological research on this issue. Materials and Methods: The trials on the combination of serum CA125 and vaginal/ pelvic US as well as the investigations on microRNA (miRNA)s, circulating tumor DNA and tumor protein 53 (TP53) variants in DNA purified from Pap smears have been critically analyzed. Results: Two large randomized trials failed to detect a reduction in ovarian cancer-related deaths in women who underwent serum CA125- and US-based screening compared to those who had no screening. The United Kingdom Collaborative Trial of Ovarian Cancer Screening reported a 39.2% higher incidence of stage I-II and 10.2% lower incidence of stage III-IV disease in women who underwent annual multimodal screening with serum CA125 and vaginal US compared to women who had no screening, but this stage shifting did not translate into a survival benefit. A longitudinal, multiple biomarker algorithm-based strategy might improve ovarian cancer detection compared with serial CA125 alone. The use of serum tumor-associated autoantibodies, circulating tumor DNA and microRNA is still investigational. The identification of TP53 clonal variants in DNA purified from Pap smears can detect early steps of serous ovarian carcinogenesis. Conclusion: The availability of sensitive next-generation sequencing-based approaches for TP53 assessment in PAP smears may allow the reliability of this genetic marker for early detection of ovarian cancer to be verified.*

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GLOBOCAN estimates of the worldwide incidence and mortality for 36 types of cancer in 185 countries have revealed 313,959 new cases of ovarian cancer and 207,252 deaths due to this malignancy in 2020 (1). The lifetime risk of ovarian cancer is 1.3% in the general population (2). It accounts for 2.5% of all female malignancies but for 5% of all cancer deaths because of its poor prognosis due to asymptomatic and silent growth and advanced stage at diagnosis, as well as to its biological aggressiveness (2, 3). It is mainly a postmenopausal tumor, with median age at diagnosis ranging from 50 to 79 years (3). Ovarian cancer includes five main types, termed high-grade serous, endometrioid, clear-cell, mucinous and low-grade serous carcinomas, which have peculiar pathological and molecular findings, and represent distinct nosological entities, with different precursor lesions, patterns of spread, response to chemotherapy and prognosis (4). High-grade serous carcinoma is the most common histological type, accounting for 70% of cases, and 80% of the patients with this malignancy are in stage III-IV at presentation. Whereas the incidence of ovarian cancer is higher among countries with a high Human Development Index, the trend of mortality tends to be opposite, with the highest rates of death in India and in Africa and with decreasing rate in Europe and North America (1, 3, 5). Two-thirds of ovarian cancer-related deaths are due to high-grade serous carcinoma. Advanced stage at presentation and residual disease after surgery are the strongest predictors of survival (6-10).

Screening for ovarian cancer in the general population is a challenging issue for oncological gynecologists. A test which leads to 10 surgical procedures for each case of ovarian cancer detected has a positive predictive value (PPV) of 10%, which can be acceptable in clinical practice (11). Since ovarian cancer has a prevalence of one in 2,500 postmenopausal women, a screening tool to obtain a PPV of 10% should have a sensitivity ≥75% for early-stage disease at 99.6% specificity (12-14). Cancer antigen-125 (CA125) is

the most reliable serum marker for this malignancy, but even in postmenopausal women its specificity is much lower than 99.6%, and therefore serum CA125 assay has usually been combined with pelvic/vaginal ultrasound (US) as screening test, with conflicting and usually disappointing results (11, 14-22). However, the evaluation of other tumor-associated antigens, tumor-associated autoantibodies, microRNAs (miRNAs) and circulating tumor DNA (ctDNA) in blood, as well as of tumor protein (*TP53)* clonal variants in DNA purified from Pap smears, appears to suggest it is possible to develop novel, non-invasive diagnostic tools for ovarian cancer. In this review, we have assessed the state of the art and the perspectives of clinical research for the screening of ovarian cancer in the general population (23-49).

CA125 and Ultrasound

Jacobs *et al*. measured serum CA125 annually in 22,000 postmenopausal British women older than 45 years, and performed pelvic US in those with an antigen value ≥30 U/ml (11). Forty-one women with abnormal US underwent surgery, which revealed an ovarian cancer in 11 and other conditions in 30. Of the 21,959 women with negative screening, eight subsequently developed ovarian cancer while 21,951 did not. Therefore, this protocol obtained a specificity of 99.9%, a PPV of 26.8%, and sensitivity of 78.6% and 57.9% at 1-year and 2-year follow-up, respectively. In a subsequent study of Jacobs *et al*., a large series of postmenopausal women aged ≥45 years were randomized to a control group or screening group (14) (Table I). Invitations to participate were sent to the 22,000 women who had participated in the previous study of screening for ovarian cancer. The patients randomized to the screening arm underwent three annual tests including serum CA125 assay followed by pelvic US. If CA125 was ≥30 U/ml and US-measured ovarian volume was ≥8.8 ml, the woman was referred to a gynecologist for additional evaluation. Of the 468 women in the screened arm with elevated CA125, 29 underwent surgery: six of them had ovarian cancer and 23 had false-positive screening results. The median survival of patients with ovarian cancer was 72.9 months in the screened group *versus* 41.8 months in the control group (p=0.0112), but there was no significant difference in cancer-related death from between the two groups.

A large number of asymptomatic postmenopausal Japanese women were randomly allocated to either a control group or intervention group which underwent an annual screening with gynecological examination, pelvic US and serum CA125 assay (17). Women with abnormal US and/or elevated serum CA125 were referred to a gynecologic oncologist. After a mean follow-up of 9.2 years, 27 cases of ovarian cancer were detected in the 41,688-screened women and eight additional cases were diagnosed outside the screening program. Ovarian cancer detection rates were 0.31:1,000 at the primary screen and ranged from 0.38:1,000 to 0.74:1,000 in subsequent screenings. Thirty-two cases of ovarian cancer were diagnosed in the 40,779 control women. Tumor was diagnosed in stage I in 63% of the cases of the screened group *versus* 38% of those of the control group but the difference was not significant.

The US Prostate, Lung, Colon, and Ovarian (PLCO) cancer screening trial enrolled 78,216 women aged 55-74 years who were randomly allocated either to a usual care arm or an intervention arm, which offered annual serum CA125 tests for 6 years and vaginal US for 4 years (18). CA125 assay result ≥35 U/ml was classified as a positive test. US examination was considered as abnormal when i) ovarian volume was >10 cm^3, ii) cyst volume was >10 cm^3, iii) there was any solid area or papillary projection extending into the cavity of any size cystic ovarian tumor, or iv) there was any mixed component within a cystic ovarian tumor. Women were followed-up for a maximum of 13 years. Overall, 212 ovarian cancer cases were detected in the intervention group and 176 in the usual care group [relative risk (RR)=1.21, 95% confidence interval (CI)=0.99-1.48], and there were 118 (3.1/10,000 person-years)deaths in the former and 100 (2.6/10,000 person-years) in the latter due to ovarian cancer (RR=1.18, 95% CI=0.82-1.71). Ovarian cancer was detected in stage III-IV in 77% of the cases of the intervention group and in 78% of those of the usual care group. All-cause mortality rates, excluding deaths from ovarian, colorectal and lung cancer, were similar in the two arms. Of the 3,285 women with false-positive results, 1,080 underwent surgery and 163 (15%) had a total of 222 major complications, including 89 infections, 63 direct surgical complications, 31 cardiovascular or pulmonary events, and 39 other complications. Therefore, the screening protocol failed to reduce ovarian cancer mortality; moreover, the surgical evaluation of false-positive cases was associated with a non-negligible rate of complications. An update of the study, with a median of 15 years of follow-up, reported that ovarian cancer-related deaths were similar in the two arms (19) (Table I). By study time period, the RR of death for the screened *versus* the unscreened group was 1.04 (95% CI=0.7-15) for years 0-7, 1.06 (95% CI=0.8-1.4) for years 7-14, and 1.09 (95% CI= 0.7-1.8) for years >14.

In a prospective cohort study, 46,101 asymptomatic women aged ≥50 years, or aged ≥25 years with a family history of ovarian cancer, received annual US screening, eventually followed by serum marker assay and surgery (20). Seventy-one ovarian cancers were detected, and of these 30 (42%) were in stage I, 15 (21%) in stage II, 26 (37%) in stage III, and none in stage IV. Disease-specific survival rates at 5, 10, and 20 years were 86%, 68%, and 65%, respectively, for women with ovarian cancer detected by screening compared with 45%, 31%, and 19%, respectively, for unscreened women

Table I. *Randomized trials comparing serum CA125 and ultrasound-based screening versus no screening for ovarian cancer in the general population.*

Authors	Study arms	Ovarian cancer deaths	RR (95% CI)
Jacobs *et al*. (14)	CA125 + pelvic US	9/10,958	2.0 (0.78-5.23)*
	No screening	18/10,977	
Buys *et al*. (18), Pinsky et al. (19)	CA 125 + pelvic US	187/34,253	1.06 (0.87-1.30)**
	No screening	176/34,304	
Jacobs *et al*. (21), Menon et al. (22)	MMS	296/50.625	0.96 (0.83-1.10)**
	USS	291/50,623	0.94 (0.82-1.08)**
	No screening	619/101,314	

RR: Relative risk; CI: confidence interval; US: ultrasound; MMS: annual multimodal screening with serum CA125 assay interpreted with risk of ovarian carcinoma algorithm; USS: vaginal ultrasound screening. **Versus* screening arm; ***versus* no screening arm.

with clinically detected ovarian cancer diagnosed in the same geographic area and treated at the same institution with the same therapeutic approach ($p<0.001$).

Serial longitudinal measurements of CA125 are more reliable than a single assay above a cut-off value for ovarian cancer screening (50-54). Skates *et al*. calculated the risk of ovarian carcinoma (ROC) based on serial CA125 assays of 33,621 serum samples collected from 9,233 postmenopausal women aged >45 years (52). All samples from patients with ovarian cancer were drawn before the clinical diagnosis of malignancy. CA125 data from each woman were summarized by the slope and intercept from a linear regression curve of log CA125 on time starting from the first serum sample, and Bayes' theorem was used to measure the ROC based on the slope and intercept of the curve and CA125 assay variability (50, 52). The area under the curve (AUC), which assessed the probability of correctly classifying a woman randomly chosen from the population, was calculated for both ROC algorithm (ROCA) and a selected cut-off value for CA125 (52). The ROCA improved the AUC compared with a fixed cut-off for CA125 antigen from 84% to 93% ($p=0.01$). Considering a target specificity of 98%, ROCA and CA125 with a selected cut-off had a sensitivity of 86% and 62%, respectively, thus suggesting that the analysis of sequential CA125 levels improved the performance of a CA125-based screening program.

Menon *et al*. randomly assigned 13,582 postmenopausal women aged ≥50 years from England, Scotland, and Wales to either a control group or a screened group with annual serum CA125 assay interpreted with the ROCA (15). Of the 6,532 screened women, 5,213 were considered to be at normal risk, 91 at high risk and 1,228 at intermediate risk of ovarian cancer. The patients with intermediate risk were recalled for another serum CA125 assay after a time interval ranging from 6 weeks to 6 months, and 53 of these women were subsequently found to have an elevated risk. All 144 women at high risk underwent vaginal US and 16 were operated on. Of these women, 11 had benign findings, one woman had adnexal relapse of breast carcinoma, another had a borderline papillary serous ovarian tumor, and three had primary ovarian cancer. No other ovarian cancer occurred during the year following the screening. Overall, the specificity and PPV for ovarian cancer were 99.8% and 19%, respectively.

ROCA was used in the United Kingdom Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) designed to assess the effect of screening on mortality in 202,638 postmenopausal women (21). Participants were randomly allocated in a 1:1:2 ratio to annual multimodal screening (MMS) with serum CA125 assay interpreted with ROCA with vaginal US as second-line test, vaginal US screening (USS), or no screening. Women with persistent abnormalities of both MMS and USS arms underwent additional clinical investigations and eventually surgery or biopsy for suspected ovarian cancer. After a median follow-up of 11.1 years, ovarian cancer was detected in 338 out of the 50,624 women (0.7%) of the MMS arm, 314 out of the 50,623 women (0.6%) of the USS arm, and 630 out of the 101,299 women (0.6%) of the no-screening arm. The sensitivity for ovarian cancer diagnosed within a year of a screening was 84% for MMS and 73% for USS, respectively. Ovarian cancer related-death occurred in 148 (0.29%), 154 (0.30%) and 347 (0.34%) women of the MMS, USS, and no-screening group, respectively. There was a non-significant reduction in mortality over years 0-14 for MMS (15%; 95% CI=−3-30%, $p=0.10$) and USS arm (11%; 95% CI=−7-27%, $p=0.21$) compared to the no-screening arm. However, between 7 and 14 years, there was a significant reduction in mortality of 23% (95% CI=1-46%) for MMS arm and a trend for reduction of 21% (95% CI=−2-42%) for the US arm. An update of the study, with a median follow-up of 16.3 years, reported that ovarian cancer was diagnosed in 522 women (1.0%) of the MMS group, 517 women (1.0%) of the USS group, and 1,016 women (1.0%) of the no-screening group, respectively (22). It is worth noting that there was a 39.2% higher incidence of stage I-II disease and 10.2% lower incidence of stage III-IV disease in the MMS group compared with no-screening group. Ovarian cancer-related deaths were

the same for the three groups (Table I). Therefore, MMS failed to reduce ovarian cancer mortality. The observation that changes in stage distribution in the MMS group did not translate into changes in death rates seems to emphasize the need for having disease-specific mortality as the primary endpoint in screening trials. Cancer cases shifted to stage I-II disease in the MMS arm may have had an intrinsically poor prognosis, not altered by earlier detection or the available treatments. A larger reduction in advanced stage incidence is likely needed for improving patient outcome. It is impossible to extrapolate these results obtained from a general population to a high-risk population, such as BRCA mutation carriers because BRCA-mutated ovarian cancer has a distinct biological and clinical behavior, with a higher response rate to chemotherapy and better clinical outcome (55-61). UKCTOCS has allowed the creation of a biobank with more than 550,000 serum samples that may be useful for the identification of novel biomarkers for ovarian cancer in the near future (22).

Tumor-associated Antigens Other than CA125, Tumor-associated Autoantibodies, miRNA and CtDNA

The analysis of several protein biomarkers in serum samples from 118 women with ovarian cancer included in the PLCO trial and 474 general population controls showed that at a fixed specificity of 95%, CA125 was the marker with the highest sensitivity (73%) followed by human epididymis protein 4 (HE4) (57%), transthyretin (47%), CA15.3 (46%) and CA72.4 (40%) (27). All these markers, except transthyretin, had similar or better sensitivity in specimens drawn within 6 months of the clinical diagnosis. The European Prospective Investigation into Cancer and Nutrition (EPIC) is a multicenter prospective cohort study designed to assess the correlation of diet, nutrition, and metabolic factors with cancer (62). CA125, HE4, CA72.4 and CA15.3 were measured in serum samples drawn from 810 women with ovarian cancer and 1,939 controls of EPIC study (31). The performance of distinguishing between cases and controls was assessed using receiver operating characteristic curves, with the AUC, as an overall measure for discriminatory ability. For samples collected within 6 months of diagnosis, the AUC was 0.92 for CA125, 0.84 for HE4, 0.77 for CA72.4 and 0.73 for CA 15.3, and marker performance decreased with longer time between serum collection and diagnosis.

Simmons *et al*. compared the sensitivity and lead time of a combination of measurement of CA125, HE4, matrix metalloproteinase-7 and CA72-4 *versus* CA125 alone in serial serum samples from 75 women who developed ovarian cancer and 547 healthy controls enrolled in the UKCTOCS trial. One or more of the complementary markers rose in 44% of the 50 CA125 screen-positive cases, without any advantage over CA125 alone in terms of lead time. The longitudinal profiles of the logarithmic biomarker concentrations were modeled with a Bayesian approach to develop single-marker longitudinal ROCA-like algorithms. The use of these algorithms showed that at a fixed 98% specificity, HE4 and CA72-4 detected 16% of the 25 CA125 screen-negative cases (43).

Serum TP53 antibodies were found in 20.9% of 86 patients with ovarian cancer in an Italian study (23), 24.8% of 113 patients in an Austrian series (24), 26.7% of 30 patients in a Japanese series (25), 39.1% of 92 patients in a Chinese study (26), and 19.5% of 220 patients of the UKCTOCS trial (32). Of these 220 patients, 164 (74.5%) were ROCA screen-positive and 56 (25.5%) were ROCA screen–negative. Thirty-four of the former (20.7%) and nine of the latter (16.1%) had elevated anti-TP53 levels. In the 34 cases with both TP53 antibody elevation and ROCA positivity, TP53 antibodies were detected 9.2 months before ROCA positivity. In 9 the cases with autoantibody positivity and ROCA negativity, TP53 antibodies rose 22.9 months before ovarian cancer diagnosis.

Very few studies have assessed the diagnostic performance of tumor-associated autoantibodies for early ovarian cancer detection (63-65). Elevated serum levels of TP53 antibodies were identified in 10.0% of the 30 patients and in 16.7% of the 12 patients with stage I-II ovarian cancer included in the Italian study (23) and in the UKCTOCS trial (32), respectively.

A finding of elevated serum interleukin-8 antibodies had a sensitivity of 65.5% and a specificity of 98% in a series of 44 patients with stage I-II ovarian cancer (63). Mean serum levels of autoantibodies against heat-shock protein 27, which is strongly associated with carcinogenesis, were significantly higher in 158 patients with ovarian carcinoma than in 80 healthy women (64).

Serum ovarian cancer-associated miRNAs represent very interesting potential novel biomarkers (34, 35, 45, 49, 66-71). Resnick *et al*. found a differential expression of serum miRNAs between patients with ovarian cancer and healthy women. Serum *miRNA-21, miRNA-92, miRNA-93, miRNA-126* and *miRNA-29a* were significantly overexpressed and serum *miRNA-155, miRNA-127* and *miRNA-99b* were significantly underexpressed in 28 patients with ovarian cancer compared with 15 healthy controls (66).

The combined assessment of serum *miRNA-142-3p, miRNA-26a-5p, let-7d-5p, miRNA-374a-5p, miRNA-766-3p, miRNA-200a-3p, miRNA-328-38p* and *miRNA-130b-3p* discriminated patients with ovarian cancer from healthy women with sensitivity of 92% and specificity of 91%, as well as patients with early-stage ovarian cancer from those with benign tumors with sensitivity of 86% and specificity of 83%, respectively (34). *miRNA-200a* is involved in ovarian carcinogenesis, *miRNA-374a* regulates cisplatin

resistance in ovarian cancer cells, and other miRNAs are functional miRNAs involved in cancer pathogenesis.

Elias *et al*. described the development of a diagnostic model for ovarian cancer using sequencing of serum miRNA (35). The neural network analysis of serum samples from 179 women selected from three independent prospective studies produced a miRNA algorithm for ovarian cancer detection with an AUC of 0.90 (35). Among the 120 women for whom serum CA125 was known, the neural network (AUC=0.93, 95% CI=0.88-0.97) outperformed the CA125 assay (AUC=0.74, 95% CI=0.65-0.83, p=0.001), with a significantly lower rate of false-positives [8/43 (18.6%) *versus* 23/43 (53.5%), p=0.002]. This neural network algorithm was tested on an external, independent dataset and revealed an excellent discriminatory power with an AUC of 0.93 (95% CI=0.81-1.00), sensitivity of 75% and specificity of 100%. The sharp decrease in serum *miR-200a* and *miR-200c* levels after debulking surgery suggested that these miRNAs are secreted actively by tumors.

A meta-analysis of 36 studies reported that the pooled sensitivity and specificity of circulating miRNAs for ovarian cancer diagnosis were 76% and 81%, respectively, with an AUC of 0.85 (95% CI=0.82-0.88) (70) (Table II).

Use of multiple miRNA assays yielded a better diagnostic reliability than using a single miRNA assay, and plasma miRNAs appeared to be better than serum miRNAs for ovarian cancer detection.

The assessment of ctDNA, which can include point mutations, microsatellite instabilities, DNA hypermethylation and loss of heterozygosity, might represent a noninvasive tool for the detection and management of ovarian cancer (33, 40, 72-74). The meta-analysis of nine studies reported that ctDNA had sensitivity of 70% and specificity of 90% for this malignancy, with an AUC of 0.89 (95% CI=0.83-0.95) (72) (Table II).

A multi-analyte test, termed CancerSEEK, assessed the plasma levels of eight protein biomarkers and mutations in ctDNA in 1,005 patients with nonmetastatic cancer of the ovary, liver, stomach, pancreas, esophagus, colorectum, lung, or breast (40) (Table II). The sensitivity for detecting ovarian cancer was 98%, with specificity >99%.

Aberrant methylation patterns of linked CpG sites analyzed in ctDNA can provide highly specific signals for cancer diagnosis (36, 73). A three DNA-methylation serum marker panel was developed and validated in women with different conditions, especially associated with serum CA125 elevation, in women with advanced ovarian cancer undergoing neoadjuvant chemotherapy, and in a subset of women enrolled in the UKCTOCS trial for whom serum samples collected up to 2 years before ovarian cancer diagnosis were available (36). This panel distinguished patients with high-grade serous ovarian cancer from healthy women, or patients with a benign pelvic mass, with a sensitivity of 41.4% and a specificity of 90.7%. For women included in the UKCTOCS trial, this panel detected 57.9% of those who developed ovarian cancer within 2 years of sample collection, with a specificity of 88.1%. The sensitivity of the test improved to 63.6% when exclusively assessing CA125-negative samples.

Table II. *Sensitivity and specificity of circulating miRNAs and circulating tumor DNA (ctDNA) for ovarian cancer.*

Authors		Sensitivity	Specificity
Zhou *et al*. (70)	Circulating miRNAs*	76%	81%
Zhou *et al*. (72)	ctDNA**	70%	90%
Cohen *et al*. (40)	CancerSEEK***	98%	>99%

*Meta-analysis of 36 studies from 16 articles with 3470 stage I-IV ovarian cancer patients and 1606 healthy controls. **Meta-analysis of nine studies including 462 patients with stage I-IV ovarian cancer and 407 controls. ***Multi-analyte test that assessed the plasma levels of eight protein biomarkers (CA125, carcinoembryonic antigen, CA19-9, prolactin, hepatocyte growth factor, osteopontin, myeloperoxidase and tissue inhibitor of metalloproteinases-1) and the presence of mutations in 1,933 distinct genomic positions of ctDNA from 1,005 patients with different malignancies, including ovarian cancer.

Tumor DNA in PAP Smears

DNA in Pap smears has been investigated for the screening of gynecological malignancies other than cervical cancer (29, 30, 39, 42, 46). By using whole-exome sequencing or targeted sequencing of frequently mutated genes, Kinde *et al*. analyzed somatic mutations in tumor tissues from 24 patients with endometrial cancer and 22 patients with ovarian cancer for whom liquid-based Pap specimens were available. These authors found the same mutations in tumor tissues and DNA from Pap smears in 100% of endometrial cancer and in 41% of ovarian cancer cases (29).

A polymerase chain reaction (PCR)-based, multiplex test, incorporating assays for mutations in 18 genes and an assay for aneuploidy, was performed on DNA purified from Pap smears to assess the genetic alterations commonly present in endometrial or ovarian cancer (39). This test, termed PapSEEK, was positive in 81% of 382 women with endometrial cancer, including 78% of those with early-stage disease, in 33% of 245 patients with ovarian cancer, including 34% of those with early-stage disease, and in 1.4% of 714 women without cancer. ctDNA was found in 43% of 83 patients with ovarian cancer for whom plasma samples were available, and the combination of PapSEEK test and plasma ctDNA assay achieved a sensitivity of 63% for this malignancy.

TP53 is the most commonly mutated gene in high-grade serous ovarian cancer, as well as in its precursor lesion, serous tubal intraepithelial carcinoma (4, 30, 75) (Table III).

Table III. *Frequency of clonal pathogenic tumor protein 53 (TP53) variants in vaginal smears from patients with ovarian cancer.*

Author	Vaginal sample	Patients, n	*TP53* mutations
Erikson *et al*. (30)	Vaginal tampon	8	3 (37.5%)
	Intact tubes	5	3 (60%)
	Tubal ligation	3	0 (0%)
Arildsen *et al*. (42)	Liquid-based PAP smear	8	6 (75.0%)
Paracchini *et al*. (46)	Brush-based and stored Pap smear	17	11 (64.7%)

Erikson *et al*. detected *TP53* mutations in all eight tumor samples from patients with high-grade serous ovarian cancer. The analysis of the DNA from a vaginal tampon placed 8-12 hours before surgery and removed in the operating room following anesthesia induction showed *TP53* mutations in three out of the five patients with intact tubes and in none of the three patients with tubal ligation. It is noteworthy that the mutation identified in vaginal DNA was the same detected in tumor tissues for all three patients (30). Arildsen *et al*. assessed *TP53* mutations by next-generation sequencing in tumor tissue samples and by droplet digital PCR in liquid-based Pap smears performed 2-7 years before diagnosis from 15 women with high-grade serous ovarian cancer. The *TP53* mutations identified in tumors were also found in vaginal smears in seven out of nine women but one of these harbored a germline mutation. Therefore, true somatic mutations were detected in six out of eight samples (Table III). An additional mutation was found in an archival Pap smear which had been collected 20 months prior to ovarian cancer diagnosis (42).

A single center Italian cohort study assessed *TP53* clonal variants with droplet digital PCR in brush-based and stored Pap smears performed in 17 patients with high-grade serous ovarian cancer up to 6 years before diagnosis (46). The same clonal somatic *TP53* variants present in tumor tissues were found in Pap smears in 64.7% of these patients (Table III), whereas these *TP53* variants were not identified in smears from healthy females. It is noteworthy that in one patient the TP53 clonal variant was detected in all three smears collected 9 days, 25 months, and 49 months before the diagnosis. Among two women with two Pap tests each, the *TP53* variant was detected 27 months and 68 months before diagnosis in one patient, and only in one of the available smears in the other patient. Clonal pathogenic *TP53* variants may identify early steps of serous ovarian carcinogenesis. These data would seem to support the mathematical model suggesting that the progression of serous tubal intraepithelial carcinoma to high-grade serous ovarian cancer may take up to 6 years (75). A weakness of the study is that archival Pap smears were not intended to be used for DNA analysis and therefore sampling procedures and storage conditions might have negatively affected DNA quality (46).

Conclusion

The two large randomized trials, PLCO and UKCTOCS, failed to detect a reduction in ovarian cancer-related deaths in patients screened with serum CA125 assay and vaginal US (18, 19, 21, 22). Whereas in the PLCO trial there was no difference in stage distribution between the two arms, the UKCTOCS trial reported a higher incidence of early disease and a lower incidence of advanced disease in the MMS group compared with no-screening group, which may have been due to the use of ROCA in this trial instead of a single CA125 value above the cut-off as in the PLCO trial (22). The unsatisfactory sensitivity of protein biomarkers for ovarian cancer screening may reflect both the relatively small size of early disease and the low biomarker expression or shedding (44). However, a longitudinal multiple biomarker algorithm-based strategy should be investigated to evaluate its ability to improve ovarian cancer detection compared with serial CA125 assays alone (43).

The use of serum tumor-associated autoantibodies, miRNA and ctDNA in ovarian cancer screening is still investigational. Future research should assess panels including several circulating miRNAs to adequately evaluate the potential role of these biomarkers in this malignancy (70).

As reported in the European Society for Medical Oncological guidelines on ovarian cancer, there are currently no standard methods for the isolation and detection of cfDNA in blood, with few studies recruiting large numbers of patients with ovarian cancer (61). Additional investigation concerning the standardization and quality control of such assays is strongly warranted before implementing this approach in clinical research and practice.

Early diagnosis of ovarian cancer is potentially achievable through the detection of *TP53* clonal variants in DNA purified from Pap smears (29, 39, 42, 46). The standardization of sampling and storage procedures of liquid-based Pap smears and the availability of highly sensitive next-generation sequencing-based approaches for *TP53* gene assessment are needed to plan a longitudinal prospective study with large numbers of patients with ovarian cancer and healthy women designed to verify the reliability of this novel diagnostic tool.

Ovarian cancer screening in the general population is still a major challenge for gynecologic oncologists, and additional clinical research on serum miRNA and ctDNA, as well as on pathogenic *TP53* variants in DNA purified from Pap smears, is strongly warranted.

Conflicts of Interest

The Authors declare no conflicts of interest.

Authors' Contributions

Conceptualization, A.G.; data curation, A.G.; writing – original draft preparation A.G.; writing – review and editing: A.G. and S.C. All Authors have read and agreed to the published version of the article.

References

1 Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A and Bray F: Global Cancer Statistics 2020: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin *71(3)*: 209-249, 2021. PMID: 33538338. DOI: 10.3322/caac.21660

2 Torre LA, Trabert B, DeSantis CE, Miller KD, Samimi G, Runowicz CD, Gaudet MM, Jemal A and Siegel RL: Ovarian cancer statistics, 2018. CA Cancer J Clin *68(4)*: 284-296, 2018. PMID: 29809280. DOI: 10.3322/caac.21456

3 Momenimovahed Z, Tiznobaik A, Taheri S and Salehiniya H: Ovarian cancer in the world: epidemiology and risk factors. Int J Womens Health *11*: 287-299, 2019. PMID: 31118829. DOI: 10.2147/IJWH.S197604

4 Prat J: New insights into ovarian cancer pathology. Ann Oncol *23(Suppl 10)*: x111-x117, 2012. PMID: 22987944. DOI: 10.1093/annonc/mds300

5 Malvezzi M, Carioli G, Rodriguez T, Negri E and La Vecchia C: Global trends and predictions in ovarian cancer mortality. Ann Oncol *27(11)*: 2017-2025, 2016. PMID: 27597548. DOI: 10.1093/annonc/mdw306

6 du Bois A, Reuss A, Pujade-Lauraine E, Harter P, Ray-Coquard I and Pfisterer J: Role of surgical outcome as prognostic factor in advanced epithelial ovarian cancer: a combined exploratory analysis of 3 prospectively randomized phase 3 multicenter trials: by the Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe Ovarialkarzinom (AGO-OVAR) and the Groupe d'Investigateurs Nationaux Pour les Etudes des Cancers de l'Ovaire (GINECO). Cancer *115(6)*: 1234-1244, 2009. PMID: 19189349. DOI: 10.1002/cncr.24149

7 Ørskov M, Iachina M, Guldberg R, Mogensen O and Mertz Nørgård B: Predictors of mortality within 1 year after primary ovarian cancer surgery: a nationwide cohort study. BMJ Open *6(4)*: e010123, 2016. PMID: 27103625. DOI: 10.1136/bmjopen-2015-010123

8 Aletti GD, Garbi A, Messori P, Achilarre MT, Zanagnolo V, Rizzo S, Alessi S, Bocciolone L, Landoni F, Biffi R, Carinelli S, Colombo N and Maggioni A: Multidisciplinary approach in the management of advanced ovarian cancer patients: A personalized approach. Results from a specialized ovarian cancer unit. Gynecol Oncol *144(3)*: 468-473, 2017. PMID: 28117100. DOI: 10.1016/j.ygyno.2017.01.017

9 Tseng JH, Cowan RA, Zhou Q, Iasonos A, Byrne M, Polcino T, Polen-De C, Gardner GJ, Sonoda Y, Zivanovic O, Abu-Rustum NR, Long Roche K and Chi DS: Continuous improvement in primary Debulking surgery for advanced ovarian cancer: Do increased complete gross resection rates independently lead to increased progression-free and overall survival? Gynecol Oncol *151(1)*: 24-31, 2018. PMID: 30126704. DOI: 10.1016/j.ygyno.2018.08.014

10 O'Shea AS: Clinical staging of ovarian cancer. Methods Mol Biol *2424*: 3-10, 2022. PMID: 34918284. DOI: 10.1007/978-1-0716-1956-8_1

11 Jacobs I, Davies AP, Bridges J, Stabile I, Fay T, Lower A, Grudzinskas JG and Oram D: Prevalence screening for ovarian cancer in postmenopausal women by CA 125 measurement and ultrasonography. BMJ *306(6884)*: 1030-1034, 1993. PMID: 8490497. DOI: 10.1136/bmj.306.6884.1030

12 Nossov V, Amneus M, Su F, Lang J, Janco JM, Reddy ST and Farias-Eisner R: The early detection of ovarian cancer: from traditional methods to proteomics. Can we really do better than serum CA-125? Am J Obstet Gynecol *199(3)*: 215-223, 2008. PMID: 18468571. DOI: 10.1016/j.ajog.2008.04.009

13 Rauh-Hain JA, Krivak TC, Del Carmen MG and Olawaiye AB: Ovarian cancer screening and early detection in the general population. Rev Obstet Gynecol *4(1)*: 15-21, 2011. PMID: 21629494.

14 Jacobs IJ, Skates SJ, MacDonald N, Menon U, Rosenthal AN, Davies AP, Woolas R, Jeyarajah AR, Sibley K, Lowe DG and Oram DH: Screening for ovarian cancer: a pilot randomised controlled trial. Lancet *353(9160)*: 1207-1210, 1999. PMID: 10217079. DOI: 10.1016/S0140-6736(98)10261-1

15 Menon U, Skates SJ, Lewis S, Rosenthal AN, Rufford B, Sibley K, Macdonald N, Dawnay A, Jeyarajah A, Bast RC Jr, Oram D and Jacobs IJ: Prospective study using the risk of ovarian cancer algorithm to screen for ovarian cancer. J Clin Oncol *23(31)*: 7919-7926, 2005. PMID: 16258091. DOI: 10.1200/JCO.2005.01.6642

16 Chu CS and Rubin SC: Screening for ovarian cancer in the general population. Best Pract Res Clin Obstet Gynaecol *20(2)*: 307-320, 2006. PMID: 16364690. DOI: 10.1016/j.bpobgyn.2005.10.012

17 Kobayashi H, Yamada Y, Sado T, Sakata M, Yoshida S, Kawaguchi R, Kanayama S, Shigetomi H, Haruta S, Tsuji Y, Ueda S and Kitanaka T: A randomized study of screening for ovarian cancer: a multicenter study in Japan. Int J Gynecol Cancer *18(3)*: 414-420, 2008. PMID: 17645503. DOI: 10.1111/j.1525-1438.2007.01035.x

18 Buys SS, Partridge E, Black A, Johnson CC, Lamerato L, Isaacs C, Reding DJ, Greenlee RT, Yokochi LA, Kessel B, Crawford ED, Church TR, Andriole GL, Weissfeld JL, Fouad MN, Chia D, O'Brien B, Ragard LR, Clapp JD, Rathmell JM, Riley TL, Hartge P, Pinsky PF, Zhu CS, Izmirlian G, Kramer BS, Miller AB, Xu JL, Prorok PC, Gohagan JK, Berg CD and PLCO Project Team: Effect of screening on ovarian cancer mortality: the prostate, lung, colorectal and ovarian (PLCO) cancer screening randomized controlled trial. JAMA *305(22)*: 2295-2303, 2011. PMID: 21642681. DOI: 10.1001/jama.2011.766

19 Pinsky PF, Yu K, Kramer BS, Black A, Buys SS, Partridge E, Gohagan J, Berg CD and Prorok PC: Extended mortality results for ovarian cancer screening in the PLCO trial with median 15years follow-up. Gynecol Oncol *143(2)*: 270-275, 2016. PMID: 27615399. DOI: 10.1016/j.ygyno.2016.08.334

20 van Nagell JR Jr, Burgess BT, Miller RW, Baldwin L, DeSimone CP, Ueland FR, Huang B, Chen Q, Kryscio RJ and Pavlik EJ:

Survival of women with Type I and II epithelial ovarian cancer detected by ultrasound screening. Obstet Gynecol *132(5)*: 1091-1100, 2018. PMID: 30303916. DOI: 10.1097/AOG.0000000000002921

21 Jacobs IJ, Menon U, Ryan A, Gentry-Maharaj A, Burnell M, Kalsi JK, Amso NN, Apostolidou S, Benjamin E, Cruickshank D, Crump DN, Davies SK, Dawnay A, Dobbs S, Fletcher G, Ford J, Godfrey K, Gunu R, Habib M, Hallett R, Herod J, Jenkins H, Karpinskyj C, Leeson S, Lewis SJ, Liston WR, Lopes A, Mould T, Murdoch J, Oram D, Rabideau DJ, Reynolds K, Scott I, Seif MW, Sharma A, Singh N, Taylor J, Warburton F, Widschwendter M, Williamson K, Woolas R, Fallowfield L, McGuire AJ, Campbell S, Parmar M and Skates SJ: Ovarian cancer screening and mortality in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): a randomised controlled trial. Lancet *387(10022)*: 945-956, 2016. PMID: 26707054. DOI: 10.1016/S0140-6736(15)01224-6

22 Menon U, Gentry-Maharaj A, Burnell M, Singh N, Ryan A, Karpinskyj C, Carlino G, Taylor J, Massingham SK, Raikou M, Kalsi JK, Woolas R, Manchanda R, Arora R, Casey L, Dawnay A, Dobbs S, Leeson S, Mould T, Seif MW, Sharma A, Williamson K, Liu Y, Fallowfield L, McGuire AJ, Campbell S, Skates SJ, Jacobs IJ and Parmar M: Ovarian cancer population screening and mortality after long-term follow-up in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): a randomised controlled trial. Lancet *397(10290)*: 2182-2193, 2021. PMID: 33991479. DOI: 10.1016/S0140-6736(21)00731-5

23 Gadducci A, Ferdeghini M, Buttitta F, Cosio S, Fanucchi A, Annicchiarico C, Gagetti O, Bevilacqua G and Genazzani AR: Assessment of the prognostic relevance of serum anti-p53 antibodies in epithelial ovarian cancer. Gynecol Oncol *72(1)*: 76-81, 1999. PMID: 9889034. DOI: 10.1006/gyno.1998.5101

24 Abendstein B, Marth C, Müller-Holzner E, Widschwendter M, Daxenbichler G and Zeimet AG: Clinical significance of serum and ascitic p53 autoantibodies in epithelial ovarian carcinoma. Cancer *88(6)*: 1432-1437, 2000. PMID: 10717627. DOI: 10.1002/(sici)1097-0142(20000315)88:6<1432::aid-cncr22>3.0.co;2-8

25 Numa F, Umayahara K, Suehiro Y, Hirakawa H, Nawata S, Suminami Y, Oga A, Ito T, Sasaki K and Kato H: Serum anti-p53 antibodies in uterine and ovarian cancer: association with dna sequence copy number abnormalities. Tumour Biol *22(3)*: 162-168, 2001. PMID: 11275794. DOI: 10.1159/000050611

26 Qiu T, Yang Q, Li XR, Yang H, Qiu LL and Wang L: Detection of serum anti-p53 antibodies from patients with ovarian cancer in China: correlation to clinical parameters. Cancer Invest *25(7)*: 563-568, 2007. PMID: 17952741. DOI: 10.1080/07357900701515434

27 Cramer DW, Bast RC Jr, Berg CD, Diamandis EP, Godwin AK, Hartge P, Lokshin AE, Lu KH, McIntosh MW, Mor G, Patriotis C, Pinsky PF, Thornquist MD, Scholler N, Skates SJ, Sluss PM, Srivastava S, Ward DC, Zhang Z, Zhu CS and Urban N: Ovarian cancer biomarker performance in prostate, lung, colorectal, and ovarian cancer screening trial specimens. Cancer Prev Res (Phila) *4(3)*: 365-374, 2011. PMID: 21372036. DOI: 10.1158/1940-6207.CAPR-10-0195

28 Zhang Q, Hu G, Yang Q, Dong R, Xie X, Ma D, Shen K and Kong B: A multiplex methylation-specific PCR assay for the detection of early-stage ovarian cancer using cell-free serum DNA. Gynecol Oncol *130(1)*: 132-139, 2013. PMID: 23623832. DOI: 10.1016/j.ygyno.2013.04.048

29 Kinde I, Bettegowda C, Wang Y, Wu J, Agrawal N, Shih IeM, Kurman R, Dao F, Levine DA, Giuntoli R, Roden R, Eshleman JR, Carvalho JP, Marie SK, Papadopoulos N, Kinzler KW, Vogelstein B and Diaz LA Jr: Evaluation of DNA from the Papanicolaou test to detect ovarian and endometrial cancers. Sci Transl Med *5(167)*: 167ra4, 2013. PMID: 23303603. DOI: 10.1126/scitranslmed.3004952

30 Erickson BK, Kinde I, Dobbin ZC, Wang Y, Martin JY, Alvarez RD, Conner MG, Huh WK, Roden RBS, Kinzler KW, Papadopoulos N, Vogelstein B, Diaz LA Jr and Landen CN Jr: Detection of somatic TP53 mutations in tampons of patients with high-grade serous ovarian cancer. Obstet Gynecol *124(5)*: 881-885, 2014. PMID: 25437714. DOI: 10.1097/AOG.0000000000000484

31 Terry KL, Schock H, Fortner RT, Hüsing A, Fichorova RN, Yamamoto HS, Vitonis AF, Johnson T, Overvad K, Tjønneland A, Boutron-Ruault MC, Mesrine S, Severi G, Dossus L, Rinaldi S, Boeing H, Benetou V, Lagiou P, Trichopoulou A, Krogh V, Kuhn E, Panico S, Bueno-de-Mesquita HB, Onland-Moret NC, Peeters PH, Gram IT, Weiderpass E, Duell EJ, Sanchez MJ, Ardanaz E, Etxezarreta N, Navarro C, Idahl A, Lundin E, Jirström K, Manjer J, Wareham NJ, Khaw KT, Byrne KS, Travis RC, Gunter MJ, Merritt MA, Riboli E, Cramer DW and Kaaks R: A prospective evaluation of early detection biomarkers for ovarian cancer in the European EPIC cohort. Clin Cancer Res *22(18)*: 4664-4675, 2016. PMID: 27060155. DOI: 10.1158/1078-0432.CCR-16-0316

32 Yang WL, Gentry-Maharaj A, Simmons A, Ryan A, Fourkala EO, Lu Z, Baggerly KA, Zhao Y, Lu KH, Bowtell D, Jacobs I, Skates SJ, He WW, Menon U, Bast RC Jr and AOCS Study Group: Elevation of TP53 autoantibody before CA125 in preclinical invasive epithelial ovarian cancer. Clin Cancer Res *23(19)*: 5912-5922, 2017. PMID: 28637689. DOI: 10.1158/1078-0432.CCR-17-0284

33 Cheng X, Zhang L, Chen Y and Qing C: Circulating cell-free DNA and circulating tumor cells, the "liquid biopsies" in ovarian cancer. J Ovarian Res *10(1)*: 75, 2017. PMID: 29132396. DOI: 10.1186/s13048-017-0369-5

34 Yokoi A, Yoshioka Y, Hirakawa A, Yamamoto Y, Ishikawa M, Ikeda SI, Kato T, Niimi K, Kajiyama H, Kikkawa F and Ochiya T: A combination of circulating miRNAs for the early detection of ovarian cancer. Oncotarget *8(52)*: 89811-89823, 2017. PMID: 29163790. DOI: 10.18632/oncotarget.20688

35 Elias KM, Fendler W, Stawiski K, Fiascone SJ, Vitonis AF, Berkowitz RS, Frendl G, Konstantinopoulos P, Crum CP, Kedzierska M, Cramer DW and Chowdhury D: Diagnostic potential for a serum miRNA neural network for detection of ovarian cancer. Elife *6*: e28932, 2017. PMID: 29087294. DOI: 10.7554/eLife.28932

36 Widschwendter M, Zikan M, Wahl B, Lempiäinen H, Paprotka T, Evans I, Jones A, Ghazali S, Reisel D, Eichner J, Rujan T, Yang Z, Teschendorff AE, Ryan A, Cibula D, Menon U and Wittenberger T: The potential of circulating tumor DNA methylation analysis for the early detection and management of ovarian cancer. Genome Med *9(1)*: 116, 2017. PMID: 29268796. DOI: 10.1186/s13073-017-0500-7

37 Kaaks R, Fortner RT, Hüsing A, Barrdahl M, Hopper M, Johnson T, Tjønneland A, Hansen L, Overvad K, Fournier A, Boutron-Ruault MC, Kvaskoff M, Dossus L, Johansson M, Boeing H, Trichopoulou A, Benetou V, La Vecchia C, Sieri S, Mattiello A, Palli D, Tumino R, Matullo G, Onland-Moret NC, Gram IT, Weiderpass E, Sánchez MJ, Navarro Sanchez C, Duell EJ, Ardanaz E, Larranaga N, Lundin E, Idahl A, Jirström K, Nodin B, Travis RC, Riboli E, Merritt M, Aune D, Terry K,

Cramer DW and Anderson KS: Tumor-associated autoantibodies as early detection markers for ovarian cancer? A prospective evaluation. Int J Cancer *143(3)*: 515-526, 2018. PMID: 29473162. DOI: 10.1002/ijc.31335

38 Elias KM, Guo J and Bast RC Jr: Early detection of ovarian cancer. Hematol Oncol Clin North Am *32(6)*: 903-914, 2018. PMID: 30390764. DOI: 10.1016/j.hoc.2018.07.003

39 Wang Y, Li L, Douville C, Cohen JD, Yen TT, Kinde I, Sundfelt K, Kjær SK, Hruban RH, Shih IM, Wang TL, Kurman RJ, Springer S, Ptak J, Popoli M, Schaefer J, Silliman N, Dobbyn L, Tanner EJ, Angarita A, Lycke M, Jochumsen K, Afsari B, Danilova L, Levine DA, Jardon K, Zeng X, Arseneau J, Fu L, Diaz LA Jr, Karchin R, Tomasetti C, Kinzler KW, Vogelstein B, Fader AN, Gilbert L and Papadopoulos N: Evaluation of liquid from the Papanicolaou test and other liquid biopsies for the detection of endometrial and ovarian cancers. Sci Transl Med *10(433)*: eaap8793, 2018. PMID: 29563323. DOI: 10.1126/scitranslmed.aap8793

40 Cohen JD, Li L, Wang Y, Thoburn C, Afsari B, Danilova L, Douville C, Javed AA, Wong F, Mattox A, Hruban RH, Wolfgang CL, Goggins MG, Dal Molin M, Wang TL, Roden R, Klein AP, Ptak J, Dobbyn L, Schaefer J, Silliman N, Popoli M, Vogelstein JT, Browne JD, Schoen RE, Brand RE, Tie J, Gibbs P, Wong HL, Mansfield AS, Jen J, Hanash SM, Falconi M, Allen PJ, Zhou S, Bettegowda C, Diaz LA Jr, Tomasetti C, Kinzler KW, Vogelstein B, Lennon AM and Papadopoulos N: Detection and localization of surgically resectable cancers with a multi-analyte blood test. Science *359(6378)*: 926-930, 2018. PMID: 29348365. DOI: 10.1126/science.aar3247

41 Guo J, Yang WL, Pak D, Celestino J, Lu KH, Ning J, Lokshin AE, Cheng Z, Lu Z and Bast RC Jr: Osteopontin, macrophage migration inhibitory factor and anti-interleukin-8 autoantibodies complement CA125 for detection of early stage ovarian cancer. Cancers (Basel) *11(5)*: 596, 2019. PMID: 31035430. DOI: 10.3390/cancers11050596

42 Arildsen NS, Martin de la Fuente L, Måsbäck A, Malander S, Forslund O, Kannisto P and Hedenfalk I: Detecting TP53 mutations in diagnostic and archival liquid-based Pap samples from ovarian cancer patients using an ultra-sensitive ddPCR method. Sci Rep *9(1)*: 15506, 2019. PMID: 31664085. DOI: 10.1038/s41598-019-51697-6

43 Simmons AR, Fourkala EO, Gentry-Maharaj A, Ryan A, Sutton MN, Baggerly K, Zheng H, Lu KH, Jacobs I, Skates S, Menon U and Bast RC Jr: Complementary longitudinal serum biomarkers to CA125 for early detection of ovarian cancer. Cancer Prev Res (Phila) *12(6)*: 391-400, 2019. PMID: 30967390. DOI: 10.1158/1940-6207.CAPR-18-0377

44 Nebgen DR, Lu KH and Bast RC Jr: Novel approaches to ovarian cancer screening. Curr Oncol Rep *21(8)*: 75, 2019. PMID: 31346778. DOI: 10.1007/s11912-019-0816-0

45 Staicu CE, Predescu DV, Rusu CM, Radu BM, Cretoiu D, Suciu N, Crețoiu SM and Voinea SC: Role of microRNAs as clinical cancer biomarkers for ovarian cancer: a short overview. Cells *9(1)*: 169, 2020. PMID: 31936634. DOI: 10.3390/cells9010169

46 Paracchini L, Pesenti C, Delle Marchette M, Beltrame L, Bianchi T, Grassi T, Buda A, Landoni F, Ceppi L, Bosetti C, Paderno M, Adorni M, Vicini D, Perego P, Leone BE, D'Incalci M, Marchini S and Fruscio R: Detection of TP53 clonal variants in Papanicolaou test samples collected up to 6 years prior to high-grade serous epithelial ovarian cancer diagnosis. JAMA Netw Open *3(7)*: e207566, 2020. PMID: 32609349. DOI: 10.1001/jamanetworkopen.2020.7566

47 Kandimalla R, Wang W, Yu F, Zhou N, Gao F, Spillman M, Moukova L, Slaby O, Salhia B, Zhou S, Wang X and Goel A: OCaMIR-a noninvasive, diagnostic signature for early-stage ovarian cancer: a multi-cohort retrospective and prospective study. Clin Cancer Res *27(15)*: 4277-4286, 2021. PMID: 34035068. DOI: 10.1158/1078-0432.CCR-21-0267

48 Biskup E, Wils RS, Hogdall C and Hogdall E: Prospects of improving early ovarian cancer diagnosis using cervical cell swabs. Anticancer Res *42(1)*: 1-12, 2022. PMID: 34969703. DOI: 10.21873/anticanres.15451

49 Zhao L, Liang X, Wang L and Zhang X: The role of miRNA in ovarian cancer: an overview. Reprod Sci, 2022. PMID: 34973152. DOI: 10.1007/s43032-021-00717-w

50 Skates SJ, Xu FJ, Yu YH, Sjövall K, Einhorn N, Chang Y, Bast RC Jr and Knapp RC: Toward an optimal algorithm for ovarian cancer screening with longitudinal tumor markers. Cancer *76(10 Suppl)*: 2004-2010, 1995. PMID: 8634992. DOI: 10.1002/1097-0142(19951115)76:10+<2004::aid-cncr2820761317>3.0.co;2-g

51 Skates S, Pauler D and Jacobs I: Screening based on the risk of cancer calculation from Bayesian hierarchical changepoint and mixture models of longitudinal markers. Journal of the American Statistical Association *96(454)*: 429-439, 2017. DOI: 10.1198/016214501753168145

52 Skates SJ, Menon U, MacDonald N, Rosenthal AN, Oram DH, Knapp RC and Jacobs IJ: Calculation of the risk of ovarian cancer from serial CA-125 values for preclinical detection in postmenopausal women. J Clin Oncol *21(10 Suppl)*: 206s-210s, 2003. PMID: 12743136. DOI: 10.1200/JCO.2003.02.955

53 Menon U, Ryan A, Kalsi J, Gentry-Maharaj A, Dawnay A, Habib M, Apostolidou S, Singh N, Benjamin E, Burnell M, Davies S, Sharma A, Gunu R, Godfrey K, Lopes A, Oram D, Herod J, Williamson K, Seif MW, Jenkins H, Mould T, Woolas R, Murdoch JB, Dobbs S, Amso NN, Leeson S, Cruickshank D, Scott I, Fallowfield L, Widschwendter M, Reynolds K, McGuire A, Campbell S, Parmar M, Skates SJ and Jacobs I: Risk algorithm using serial biomarker measurements doubles the number of screen-detected cancers compared with a single-threshold rule in the United Kingdom collaborative trial of ovarian cancer screening. J Clin Oncol *33(18)*: 2062-2071, 2015. PMID: 25964255. DOI: 10.1200/JCO.2014.59.4945

54 Blyuss O, Burnell M, Ryan A, Gentry-Maharaj A, Mariño IP, Kalsi J, Manchanda R, Timms JF, Parmar M, Skates SJ, Jacobs I, Zaikin A and Menon U: Comparison of longitudinal CA125 algorithms as a first-line screen for ovarian cancer in the general population. Clin Cancer Res *24(19)*: 4726-4733, 2018. PMID: 30084833. DOI: 10.1158/1078-0432.CCR-18-0208

55 Alsop K, Fereday S, Meldrum C, deFazio A, Emmanuel C, George J, Dobrovic A, Birrer MJ, Webb PM, Stewart C, Friedlander M, Fox S, Bowtell D and Mitchell G: BRCA mutation frequency and patterns of treatment response in BRCA mutation-positive women with ovarian cancer: a report from the Australian Ovarian Cancer Study Group. J Clin Oncol *30(21)*: 2654-2663, 2012. PMID: 22711857. DOI: 10.1200/JCO.2011.39.8545

56 Dann RB, DeLoia JA, Timms KM, Zorn KK, Potter J, Flake DD 2nd, Lanchbury JS and Krivak TC: BRCA1/2 mutations and expression: response to platinum chemotherapy in patients with advanced stage epithelial ovarian cancer. Gynecol Oncol *125(3)*: 677-682, 2012. PMID: 22406760. DOI: 10.1016/j.ygyno.2012.03.006

57 Safra T, Borgato L, Nicoletto MO, Rolnitzky L, Pelles-Avraham S, Geva R, Donach ME, Curtin J, Novetsky A, Grenader T, Lai WC, Gabizon A, Boyd L and Muggia F: BRCA mutation status and determinant of outcome in women with recurrent epithelial ovarian cancer treated with pegylated liposomal doxorubicin. Mol Cancer Ther *10(10)*: 2000-2007, 2011. PMID: 21835933. DOI: 10.1158/1535-7163.MCT-11-0272

58 Sun C, Li N, Ding D, Weng D, Meng L, Chen G and Ma D: The role of BRCA status on the prognosis of patients with epithelial ovarian cancer: a systematic review of the literature with a meta-analysis. PLoS One *9(5)*: e95285, 2014. PMID: 24788697. DOI: 10.1371/journal.pone.0095285

59 Monk BJ, Lorusso D, Italiano A, Kaye SB, Aracil M, Tanović A and D'Incalci M: Trabectedin as a chemotherapy option for patients with BRCA deficiency. Cancer Treat Rev *50*: 175-182, 2016. PMID: 27710871. DOI: 10.1016/j.ctrv.2016.09.009

60 Gadducci A and Guerrieri ME: PARP inhibitors alone and in combination with other biological agents in homologous recombination deficient epithelial ovarian cancer: From the basic research to the clinic. Crit Rev Oncol Hematol *114*: 153-165, 2017. PMID: 28477743. DOI: 10.1016/j.critrevonc.2017.04.006

61 Colombo N, Sessa C, du Bois A, Ledermann J, McCluggage WG, McNeish I, Morice P, Pignata S, Ray-Coquard I, Vergote I, Baert T, Belaroussi I, Dashora A, Olbrecht S, Planchamp F, Querleu D and ESMO-ESGO Ovarian Cancer Consensus Conference Working Group: ESMO-ESGO consensus conference recommendations on ovarian cancer: pathology and molecular biology, early and advanced stages, borderline tumours and recurrent disease†. Ann Oncol *30(5)*: 672-705, 2019. PMID: 31046081. DOI: 10.1093/annonc/mdz062

62 Bingham S and Riboli E: Diet and cancer – the European Prospective Investigation into Cancer and Nutrition. Nat Rev Cancer *4(3)*: 206-215, 2004. PMID: 14993902. DOI: 10.1038/nrc1298

63 Lokshin AE, Winans M, Landsittel D, Marrangoni AM, Velikokhatnaya L, Modugno F, Nolen BM and Gorelik E: Circulating IL-8 and anti-IL-8 autoantibody in patients with ovarian cancer. Gynecol Oncol *102(2)*: 244-251, 2006. PMID: 16434085. DOI: 10.1016/j.ygyno.2005.12.011

64 Olejek A, Damasiewicz-Bodzek A, Bodzek P, Wielkoszyński T, Zamłyński J, Stołtny P and Skutil M: Concentrations of antibodies against heat shock protein 27 in the sera of women with ovarian carcinoma. Int J Gynecol Cancer *19(9)*: 1516-1520, 2009. PMID: 19955928. DOI: 10.1111/IGC.0b013e3181bf425b

65 Fortner RT, Damms-Machado A and Kaaks R: Systematic review: Tumor-associated antigen autoantibodies and ovarian cancer early detection. Gynecol Oncol *147(2)*: 465-480, 2017. PMID: 28800944. DOI: 10.1016/j.ygyno.2017.07.138

66 Resnick KE, Alder H, Hagan JP, Richardson DL, Croce CM and Cohn DE: The detection of differentially expressed microRNAs from the serum of ovarian cancer patients using a novel real-time PCR platform. Gynecol Oncol *112(1)*: 55-59, 2009. PMID: 18954897. DOI: 10.1016/j.ygyno.2008.08.036

67 Marchini S, Cavalieri D, Fruscio R, Calura E, Garavaglia D, Fuso Nerini I, Mangioni C, Cattoretti G, Clivio L, Beltrame L, Katsaros D, Scarampi L, Menato G, Perego P, Chiorino G, Buda A, Romualdi C and D'Incalci M: Association between miR-200c and the survival of patients with stage I epithelial ovarian cancer: a retrospective study of two independent tumour tissue collections. Lancet Oncol *12(3)*: 273-285, 2011. PMID: 21345725. DOI: 10.1016/S1470-2045(11)70012-2

68 Leslie M: Cell Biology. NIH effort gambles on mysterious extracellular RNAs. Science *341(6149)*: 947, 2013. PMID: 23990535. DOI: 10.1126/science.341.6149.947

69 Gadducci A, Sergiampietri C, Lanfredini N and Guiggi I: MicroRNAs and ovarian cancer: the state of art and perspectives of clinical research. Gynecol Endocrinol *30(4)*: 266-271, 2014. PMID: 24479883. DOI: 10.3109/09513590.2013.871525

70 Zhou Q, Zuo MZ, He Z, Li HR and Li W: Identification of circulating microRNAs as diagnostic biomarkers for ovarian cancer:A pooled analysis of individual studies. Int J Biol Markers: 1724600818766500, 2018. PMID: 29683066. DOI: 10.1177/1724600818766500

71 Ghafouri-Fard S, Shoorei H and Taheri M: miRNA profile in ovarian cancer. Exp Mol Pathol *113*: 104381, 2020. PMID: 31954715. DOI: 10.1016/j.yexmp.2020.104381

72 Zhou Q, Li W, Leng B, Zheng W, He Z, Zuo M and Chen A: Circulating cell free DNA as the diagnostic marker for ovarian cancer: a systematic review and meta-analysis. PLoS One *11(6)*: e0155495, 2016. PMID: 27253331. DOI: 10.1371/journal.pone.0155495

73 Bettegowda C, Sausen M, Leary RJ, Kinde I, Wang Y, Agrawal N, Bartlett BR, Wang H, Luber B, Alani RM, Antonarakis ES, Azad NS, Bardelli A, Brem H, Cameron JL, Lee CC, Fecher LA, Gallia GL, Gibbs P, Le D, Giuntoli RL, Goggins M, Hogarty MD, Holdhoff M, Hong SM, Jiao Y, Juhl HH, Kim JJ, Siravegna G, Laheru DA, Lauricella C, Lim M, Lipson EJ, Marie SK, Netto GJ, Oliner KS, Olivi A, Olsson L, Riggins GJ, Sartore-Bianchi A, Schmidt K, Shih lM, Oba-Shinjo SM, Siena S, Theodorescu D, Tie J, Harkins TT, Veronese S, Wang TL, Weingart JD, Wolfgang CL, Wood LD, Xing D, Hruban RH, Wu J, Allen PJ, Schmidt CM, Choti MA, Velculescu VE, Kinzler KW, Vogelstein B, Papadopoulos N and Diaz LA Jr: Detection of circulating tumor DNA in early- and late-stage human malignancies. Sci Transl Med *6(224)*: 224ra24, 2014. PMID: 24553385. DOI: 10.1126/scitranslmed.3007094

74 Campan M, Moffitt M, Houshdaran S, Shen H, Widschwendter M, Daxenbichler G, Long T, Marth C, Laird-Offringa IA, Press MF, Dubeau L, Siegmund KD, Wu AH, Groshen S, Chandavarkar U, Roman LD, Berchuck A, Pearce CL and Laird PW: Genome-scale screen for DNA methylation-based detection markers for ovarian cancer. PLoS One *6(12)*: e28141, 2011. PMID: 22163280. DOI: 10.1371/journal.pone.0028141

75 Labidi-Galy SI, Papp E, Hallberg D, Niknafs N, Adleff V, Noe M, Bhattacharya R, Novak M, Jones S, Phallen J, Hruban CA, Hirsch MS, Lin DI, Schwartz L, Maire CL, Tille JC, Bowden M, Ayhan A, Wood LD, Scharpf RB, Kurman R, Wang TL, Shih IM, Karchin R, Drapkin R and Velculescu VE: High grade serous ovarian carcinomas originate in the fallopian tube. Nat Commun *8(1)*: 1093, 2017. PMID: 29061967. DOI: 10.1038/s41467-017-00962-1

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