High-Grade Serous Ovarian Cancer

Subjects: Oncology | Obstetrics & Gynaecology

Contributor: Jacek Wilczyński, Edyta Paradowska, Miłosz Wilczyński

High-grade serous ovarian cancer (HGSOC) is the most lethal tumor of the female genital tract. Despite extensive studies and the identification of some precursor lesions like serous tubal intraepithelial cancer (STIC) or the deviated mutational status of the patients (*BRCA* germinal mutation), the pathophysiology of HGSOC and the existence of particular risk factors is still a puzzle. Moreover, a lack of screening programs results in delayed diagnosis, which is accompanied by a secondary chemo-resistance of the tumor and usually results in a high recurrence rate after the primary therapy. Therefore, there is an urgent need to identify the substantial risk factors for both predisposed and low-risk populations of women, as well as to create an economically and clinically justified screening program.

Keywords: ovarian cancer ; risk factors ; diagnosis ; prediction ; biomarkers ; liquid biopsy ; circulating tumor cells ; circulating tumor DNA

1. Risk Factors for HGSOC

1.1. Epidemiological Risk Factors

The risk factors that have been commonly accepted and cited in many publications include age (usually less than 45 years), more ovulatory cycles during a lifetime [1], early menarche and late menopause, nulliparity, a lack of breastfeeding [2][3], the non-usage of oral hormonal contraceptives [4], the use of estrogen menopausal replacement therapy [5], diabetes, obesity, and cigarette smoking [1]. The observation that tubal ligation, salpingo-oophorectomy, and hysterectomy could protect against HGSOC suggests the ascending infection and the use of talc on examination gloves or for perineal hygiene as a risk factor [3][6]. A diet rich in animal proteins contains xeno-estrogens, which have carcinogenic potential [7]. High levels of serum lipids resulting from increased fat consumption stimulate leptin secretion by fatty tissue, stimulating the release of gonadotropins. This mechanism could result in disturbed re-epithelialization of ovaries and predisposes one to abnormal epithelium proliferation [8]. A lower intake of polyunsaturated omega-3 fatty acids and a high consumption of trans-fats were also associated with an increased risk of ovarian cancer [9]. However, epidemiological analysis revealed that only about 10% of ovarian cancer cases could be attributed to the presence of the above-mentioned risk factors [10].

1.2. Gene Mutations

Genetic studies have brought knowledge about other risk factors. The most discussed of them is the presence of the familiar germline *BRCA1/2* mutation. It is estimated that about 3,5% of ovarian cancer patients have germline *BRCA* mutations, and 10–20% of ovarian cancers could be associated with these mutations [11][12]. The risk of ovarian cancer in this group of patients varies from 27% to 44% in the *BRCA2*-mutated and *BRCA1*-mutated patients, respectively [13]. For comparison, the lifetime risk of ovarian cancer in non-mutated populations does not exceed 1.5% [1]. Except for high-penetrance (high-risk) *BRCA* alleles, several low-penetrance (low-risk) alleles could account for the increased risk of ovarian cancer, including mutations in BRCA1-interacting helicase 1 (*BRIP1*), RAD51 Paralog C (*RAD51C*), *RAD51D*, and partner and localizer of BRCA2 (*PALB2*) genes, with the odds ratio of ovarian cancer being 14.1 for *BRIP1*, 5.2 for *RAD1C*, and 12.0 for *RAD1D* mutation and a relative risk of 2.9 for *PALB2*, respectively [14][15]. This risk ratio translates to a lifetime risk of 5–12% [16]. Both high- and low-risk mutations account for an ordinary risk of 40% for ovarian cancer [17]. The association of other low-penetrance allele mutations was also considered for ATM serine/threonine kinase (*ATM*), checkpoint kinase 2 (*CHEK2*), and BRCA1-associated RING domain 1 (*BARD1*) genes. According to the National Comprehensive Cancer Network (NCCN v.2.2022.), only the mutation of the *ATM* gene could influence the increase in the risk of ovarian cancer. It should be considered for familiar screening [18]. All these genes are connected with the homologous recombination (HR) pathway of DNA repair, which is seriously disturbed in HGSOC. Therefore, patients with the presence of one high-risk mutation or at least two low-risk mutations may benefit from therapies targeting dysfunctional genes, like poly (ADP-ribose) polymerase (PARP) inhibitors and drugs of similar mechanisms of action [19]. Lynch syndrome, also called hereditary nonpolyposis colorectal cancer syndrome (HNPCC), is associated with a disturbed DNA mismatch repair system and microsatellite instability due to the MutL protein homolog 1 (*MLH1*) and MutS

homolog 2 (*MSH2*) mutations [20]. A clinical sign of the syndrome is colorectal cancer associated with endometrial and ovarian cancers. Ovarian cancer risk in women with Lynch syndrome is 6–8% and is higher in the presence of *MSH2* and *MSH6* mutations [21]. Women with Lynch syndrome-associated ovarian cancer are diagnosed earlier, usually have non-serous histology, and, in 22% of cases, ovarian tumors exist with synchronous endometrial cancer [22][23]. Li–Fraumeni syndrome is an autosomal dominant syndrome determined by heterozygous germline mutations in the tumor suppressor gene *TP53.* Less frequent tumors (15%) associated with Li–Fraumeni syndrome include ovarian cancer and occur at an earlier age compared to the onset age of sporadic ovarian cancer [24][25]. Tumors are typically characterized by worse survival, increased chemo-resistance, and a high relapse rate [26].

1.3. Gene Polymorphisms

The analysis of single nucleotide polymorphisms (SNPs) in many genes regulating ovarian cancer biology has been performed. Although the *CHEK2* mutations were not confirmed as significant regarding the risk of ovarian cancer, other studies indicated that *CHEK2* SNP rs17507066 had a significant association with the risk of HGSOC [18]. In a group of 587 studied patients with epithelial ovarian cancer, SNPs at *PIK3CA* rs9838117 and *ERBB2* rs1058808 loci associated with cancer cell signaling were found to be correlated with the risk of cancer [27]. In the panel of genes related to ovarian cancer relapse, SNP analysis indicated that the male-specific lethal-1 homolog (*MSL1*) SNP rs7211770 was associated with a decreased risk of HGSOC (OR 0.81). At the same time, tumor suppressor HEXIM P-TEFb Complex Subunit 1 (*HEXIM1*) SNP rs1053578 was correlated with an increased risk of ovarian cancer (OR 1.4) [28]. The transcription elongation factor A (SII)-like 7 (*TCEAL7*) gene is epigenetically down-regulated in ovarian cancers, and SNPs rs5987515, rs5987724, and rs5945971 were significantly associated with a reduced risk of HGSOC [29]. Micro RNA (miR) miR-196a-2 locus polymorphism is observed in several malignancies, and studies have indicated that the miR-196a-2 rs11614913 CC genotype may increase the risk of ovarian cancer and enhance cell invasion [30]. The hephaestin gene (*HEPH*) encoding the protein responsible for iron transport regulates the proliferation and apoptosis of cancer cells and facilitates reactive oxygen species (ROS) production [31]. The *HEPH* SNP rs17216603 was associated with a reduced risk of HGSOC (OR 0.81) [32]. Somatic mutations in the retinoblastoma-1 (*RB1*) gene have been observed in several malignancies. The *RB1* rs2854344 and rs4151620 polymorphism have been associated with a reduced risk of ovarian cancer [30]. The insulin-like growth factor binding protein 3 (*IGFBP3*) gene functions as a low-penetrance onco-suppressor gene and regulates the interaction between insulin growth factor-1 (IGF-1) and its receptor [33]. IGF-1 enhances tumor growth and inhibits cell apoptosis. The *IGFBP3* SNP rs2270628 was associated with increased IGF1 plasma levels and higher ovarian cancer risk [34]. The epigenetic regulation of cancer growth is, among other things, regulated by DNA methylation provided by DNA methyltransferases encoded by *DNMT* genes. The *DNMT1* rs2228611 and rs759920 SNPs were associated with an increased risk of ovarian cancer development (OR 1.8 and OR 1.9, respectively) [35]. The inflammatory pathways are activated in many cancers and are important in ovarian cancer. The interleukin-1α (*IL1A*) gene SNP rs17561 was associated with a reduced risk of endometroid, mucinous, and clear-cell ovarian cancers but not HGSOC, indicating the genetic diversity of ovarian epithelial tumors [36]. Toll-like receptors (TLRs) are engaged in the inflammatory pathways, and their activation in ovarian cancer is closely related to cancer aggressiveness, chemo-resistance, and adverse clinical outcomes [37][38]. The interaction between TLR4 and MyD88, the molecule considered to be the marker of stem cells in ovarian cancer, contributes to inflammation in the tumor environment and enhances its aggressive phenotype [39]. Recent findings suggest that the *TLR4* Asp299Gly polymorphism could be a genetic risk factor for the development of ovarian cancer [40]. Class II human leukocyte antigens (HLA) regulate interactions between cancer cells and host immune responses represented by tumor-infiltrating lymphocytes (TILs). The intercellular adhesion molecule-1 (ICAM-1) also plays an essential function in immune reactions and cell–cell contact in the tumor environment. The significant association of *HLA-DP* rs3077 AA, *HLA-DQ* rs3920 GG, *ICAM-1* rs1437 CC, and CT genotypes with increased risk of ovarian cancer (OR 43, 6, 25, and 2.6, respectively) was confirmed. Moreover, *HLA-DQ* rs3920 and *ICAM-1* rs1437 alleles varied significantly among borderline and malignant types of ovarian cancer, highlighting once more the need for an individual approach to different ovarian cancer subtypes [41]. Recently, calculating polygenic risk scores for ovarian cancer has improved risk stratification and may be used in clinical prevention programs [42].

1.4. Microbiome

1.4.1. Viral Infections

It is estimated that microbial infections account for even 20% of cancer cases, and several viruses have been involved in carcinogenesis directly and indirectly through the stimulation of chronic inflammation and immunosuppression [43][44]. Studies confirming the presence of viruses in ovarian tumors have been noticed since 1992 when the presence of human papillomavirus (HPV) 16 and 18 DNA was confirmed in ovarian cancer tissue [45]. Investigations of HGSOC confirmed the presence of HPV 16 and 33 DNA in 10.5% of samples [46], HPV 6 DNA in 25% [47], HPV 16 DNA in 5.7% [48], and

cytomegalovirus (CMV) DNA in 40–50% of samples [47][49]. HPV and CMV co-infection was observed in almost two-thirds of malignant ovarian cancer tissues containing mostly HGSOC, and the presence of viruses was also noticed in fallopian tubes [50]. Generally, in advanced ovarian cancer, the predominant genotypes are high-risk HPV 16 and 18, similar to cervical cancer [51]. The integration of HPV oncogenes *E6* and *E7* followed by the degradation of p53 and Rb proteins triggers carcinogenesis [52] and could hypothetically account for the origin of superficial tubal intraepithelial cancer (STIC), which precedes HGSOC. Of the genes that could be affected by HPV 16 insertions, more than half were found to be associated with malignant solid tumors, and in the case of HPV 18, two genes associated with malignancy in solid tumors were affected by genomic integration [53]. Similarly, *Herpes* virus insertions were also noticed at the sites of genes related to epithelial cancer or different solid cancers [53]. The CMV immediate-early (IE) and late tegument (pp65) proteins were abundant in ovarian cancer tissues and correlated with worse prognosis [49]. CMV proteins can inhibit the cycle arrest functions of p53 and modulate the function of many key signaling pathways, including Wnt/β-catenin, PI3K/AKT, NF-κB, and STAT3, thus stimulating proliferation, angiogenesis, inflammation, and immune escape [54][55][56][57]. Epstein–Barr virus (EBV) is a herpes virus associated with several human malignancies [58]. The presence of EBV DNA was confirmed in 7.8% of ovarian cancer samples, but no EBV DNA presence was noticed specifically in HGSOC samples [59][60]. Compared to controls, ovarian cancer is also characterized by a different composition of viral signatures, with *Anelloviridae*, *Astroviridae*, *Birnaviridae*, *Bornaviridae*, *Hepadnaviridae*, *Iridoviridae*, *Paramyxoviridae*, and *Rhabdoviridae* found in significant levels exclusively in ovarian cancer samples [53]. Similarly, the *Poxviridae* family and Merkel cell *Polyomavirus* were also over-represented in ovarian cancer tissues [53].

1.4.2. Bacterial Microbiome

In addition to viruses, a bacterial microbiome could account for ovarian cancer risk. Changes to microbiome compartments (such as vaginal, peritoneal, or tumor tissue compartments) may play an essential role in the pathogenesis of ovarian malignancy. Such a shift in the microbiome in the case of neoplastic diseases is termed oncobiosis. Oncobiosis-related interactions may influence cancer hallmarks, i.e., cellular metabolism, or induce gene expression changes, eventually leading to larger-scale events, such as epithelial-to-mesenchymal transition or angiogenesis [61]. Both bacteria and bacterial metabolites modulate the actions of the immune system. The oncobiotic transformation may cause a tolerogenic state that reduces the elimination of early cancer cells. However, immunogenic actions related to the dysbiotic microbiome may also lead to tumorigenic inflammation, the induction of oxidative stress, and a higher risk of mutations [61]. The oncobiotic microbiome in the peritoneum or tumor tissues differs from the healthy state. Several authors have suggested that the immunogenic character of the microbiome is associated with an increase in the presence of Gram-negative bacteria [62][63][64].

The observation that a history of pelvic inflammatory disease (PID) increased the risk of ovarian borderline tumors and HGSOC suggested a link between genital tract infection and ovarian cancer [65][66]. Another study showed that a history of more than five episodes of PID was correlated with a 2.5-times more significant risk of ovarian cancer in women aged over 35 years [67]. One of the considerable causes of PID is *Chlamydia trachomatis* infection [68]. The DNA of *Chlamydia* was found in 17% of ovarian cancer samples, and chlamydial heat shock protein-60 (HSP-60) was detected more frequently in HGSOC samples than in benign ovarian tumors [69]. The presence of chlamydial plasmid-encoded Pgp3 (virulence factor in genital tract) antibody was associated with a doubled risk of ovarian cancer [70]. The mechanistic explanation for the relation between *Chlamydia* infection and ovarian cancer is based on the observations that infection provokes oxidative DNA damage, the down-regulation of p53 function, and induces DNA double-strand breaks [71][72]. Moreover, chlamydial infection causes an epithelial-to-mesenchymal transition (EMT) in host cells and increases the stemness and CpG methylation of genes. All of these actions may contribute to promoting HGSOC cancer [73][74]. Menopausal status is one of the factors influencing the composition of cervicovaginal microbiota. In healthy premenopausal women, a *Lactobacillus*-dominated microbiota was observed. At the same time, after menopause, it shifted to a highly diverse bacterial species dominated by *Propionibacterium*, *Corynebacterium*, and *Staphylococcus*, similar to skin bacterial composition [75][76]. In ovarian cancer, a *Lactobacillus*-poor and highly diversified microbiota mimicking the post-menopausal environment was described, regardless of the menopausal status [75]. Surprisingly, it was discovered that women with the *BRCA1* mutation had *Lactobacillus*-depleted cervicovaginal microbiota even before the onset of ovarian cancer [77]. In the study performed by Banerjee et al. [53], two predominant bacterial phyla were detected, mainly *Proteobacteria* and *Firmicutes*, present in 52% and 22% of ovarian cancer samples, respectively. Similarly, the *Proteobacteria*/*Firmicutes* ratio was significantly increased in ovarian cancer samples [78]. This study confirmed that ovarian cancer samples had much more diversified microbiota than controls.

The upper genital tract was in the past thought to be a sterile environment; however, recent studies contradict these notions. The ovarian follicular fluid of patients subjected to in vitro fertilization contained bacteria such as *Actinomyces*, *Staphylococcus*, *and Bifidobacterium* [79]. The endometrial cavity also indicates the presence of bacteria, with *Bacteroides*

being a dominant resident of the non-pregnant endometrium [80]. A microbiome was also confirmed in the fallopian tubes, fimbriae, and ovarian surface epithelium. *Firmicutes*, *Proteobacteria*, *Actinobacteria*, and *Bacteroidetes* phyla were identified in the upper genital tract, with *Proteobacteria* being the most abundant while *Firmicutes* were the least common microbiota, respectively. There were also differences in microbiota distribution between tubes, fimbriae, and ovaries [81]. In ovarian cancer, the most abundant microbiota were *Ralstonia*, *Mycobacterium*, and *Variovorax*, and the abundances of several taxa were reduced in cancer compared to control samples, indicating the occurrence of bacterial dysbiosis in ovarian cancer [81].

The probable influence of the gut microbiome on polycystic ovary syndrome and endometriosis or chronic pelvic pain has been postulated [82][83]. The impact of the gut microbiome on the formation and development of ovarian cancer is much more hypothetical; however, the connections between gut microbiome and intestinal estrobolome could explain this dependency. Firstly, the gut microbiome disturbs the entero-hepatic circulation of estrogen [84]. Secondly, the microbiome interferes with the secretion of β-glucuronidase, an enzyme responsible for the degradation of the active forms of estrogen [85]. Through both mechanisms, the gut microbiome up-regulates the estrogen level and induces carcinogenesis with β-glucuronidase [86]. A recent study on a mouse model of HGSOC could confirm the role of the gut microbiome in ovarian cancer. Control and antibiotic-treated mice were stimulated by tamoxifen to build up malignant ovarian tumors. After one year of observation, it was found that antibiotic-treated mice had significantly fewer and less advanced tumors. The changes in gut microbiota following antibiotic therapy included the increase in *Bacteroidales* species, which are negatively correlated with cancer, accompanied by the reduction or elimination of cancer-associated *Prevotella*. It seems that *Bacteroidales* in the fecal microbiota might prevent the recolonization of cancer-associated *Prevotella* and further inhibit HGSOC development [27].

1.4.3. Fungal and Parasitic Infections

The fungal and parasitic signatures have also been found to differentiate between ovarian cancer patients and control groups. The signatures of *Cladosporium*, *Pneumocystis*, *Acremonium*, *Malassezia*, and *Pleistophora* were detected in all ovarian cancer samples, whereas signatures of *Rhizomucor*, *Rhodotorula*, *Alternaria*, and *Geotrichum* were detected in 95% of cancer samples [53]. Parasitic signatures in ovarian cancer samples were significantly more diversified compared to controls. They revealed the presence of genetic material from *Dipylidium*, *Trichuris*, *Leishmania*, and *Babesia* in all samples, as well as signatures of *Trichinella*, *Ascaris*, and *Trichomonas* in more than 95% of cancer samples [53].

2. Prediction of HGSOC

2.1. Tumor Markers

2.1.1. CA125

The classic and most widely used tumor marker for HGSOC is CA125, a glycoprotein encoded by the *MUC16* gene [87]. CA125 is detected in serum samples of more than 80% of ovarian cancer patients; however, its accuracy for detecting early-stage ovarian cancer is limited [88][89]. The low sensitivity of CA125 (50–60%) for detecting early-stage ovarian cancer arises because about 50% of such patients do not have elevated CA125 levels [90]. The specificity of CA125 is also relatively low (70–80%), and about 6/10 patients with increased CA125 levels do not have ovarian cancer but have different causes, like other malignancies and benign conditions (menstruation, pregnancy, benign ovarian tumors, uterine fibroids, endometriosis, adenomyosis, pelvic inflammatory disease, and liver diseases) [91]. To improve CA125 accuracy, different approaches have been developed. The detection of CA125-containing exosomes was shown to improve specificity, sensitivity, and AUC [92]. Detecting the glycoforms of CA125 is another strategy to enhance the efficacy of CA125 testing. CA125 is a highly glycosylated protein containing many Thomsen-nouveau (Tn) antigens, which are significantly up-regulated in ovarian cancer cells but have low expression in normal tissue. The combined use of CA125-Tn improved the diagnosis specificity in patients over 45 years [93]. Another form of Tn antigen, the sialyl-Tn antigen (STn), is exclusively abundant in cancer cells but has limited concentration in normal cells. The STn serum levels were increased by around 50%, 10%, and 4% in ovarian cancer patients, endometriosis, and healthy controls, respectively [94].

2.1.2. HE4

Human Epididymis Secretory Protein 4 (HE4) has been introduced to ovarian cancer diagnosis. Compared with CA125, HE4 is less frequently affected by benign gynecological conditions, especially endometriosis [95]. HE4 provides a specificity of 96% and a sensitivity of 67% for the detection of ovarian cancer [96]. HE4 enabled the diagnosis of more than half of ovarian tumors not expressing CA125. In early-stage ovarian cancer, the sensitivity and specificity of HE4 were 0.64 and 0.87, respectively, and HE4 performed better than CA125 concerning the specificity (97% vs. 67%) [97][98]. However, similarly to CA125, HE4 is not specific to ovarian cancer and may be increased in other malignancies

(endometrial, lung, and breast cancers) and adenomyosis [95][97]. The diagnostic accuracy of HE4 is modified by both the age and menopausal status of the patients. Therefore, HE4 performed better in postmenopausal women than in premenopausal women but had decreased specificity and sensitivity in older women [99][100]. As with CA125, impaired renal function could be a source of significant mistakes in HE4 interpretation [100].

2.1.3. CA-72-4

Glycoprotein CA72-4 is elevated in ovarian cancer, but contrary to CA125, it is not affected by the menstrual cycle, pregnancy, or endometriosis [101][102]. Its increased levels were detected in mucinous and clear-cell cancers, where HE4 usually was not changed. Therefore, CA72-4 is a valuable additional marker in the cases missed by standard diagnostic markers [103].

2.1.4. FOLR1

Folate receptor-α (FOLR1) is over-expressed in several malignant cancers, including 76% of patients with HGSOC [104] [105]. Its serum concentration significantly increases in malignant compared to benign ovarian tumors and healthy controls [106]. The concentration of FOLR1 depends on the clinical stage of the cancer and its histology, grade, and size; therefore, FOLR1 serum levels are much higher in the case of aggressive and advanced tumors of the HGSOC type while being lower in mucinous and early-stage tumors [107].

2.1.5. TTR

Transthyretin (TTR) is a protein that transports thyroxine and retinol to the liver. Contrary to the previously discussed markers, its serum concentration is decreased in ovarian cancer. TTR performed better than CA125 and HE4 in detecting early-stage ovarian cancer; however, because of low sensitivity, it proved more efficient when combined with other biomarkers [108].

2.1.6. Multivariate Index Assays

Further need to improve diagnostic accuracy brought about the implementation of multivariate index assays. One of the first such approaches was the introduction of serial CA125 measurements and their use in calculating the ROCA (Risk of Ovarian Cancer Algorithm). The ROCA used with transvaginal ultrasound (TVUS) in patients with increased serial CA125 levels improved sensitivity to 85% for earlier detection [109]. The UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) revealed that longitudinal CA125 testing combined with annual TVUS screening improved the recognition capability for early-stage ovarian cancer by 47%. Still, it was not accompanied by a significant decrease in mortality [110].

The Risk of Ovarian Malignancy Index (ROMI) is a multivariate index assay based on serum levels of CA125, HE4, and thymidine kinase-1 (TK1). It showed higher specificity and sensitivity than ROMA in premenopausal and postmenopausal women [111].

The subsequent multivariate index assay is OVA1, which combines serum biomarkers CA125, transthyretin, transferrin, beta-2 microglobulin, and apolipoprotein A-1 to calculate a risk score for ovarian cancer. Compared to CA125 alone, OVA1 performs better concerning the sensitivity and identification of 60–80% of early-stage ovarian cancer cases missed by CA125 serum measurements [112][113]. OVERA was created as the second-generation test for OVA1 and combined CA125, HE4, apolipoprotein A1, transferrin, and follicle-stimulating hormone for screening patients with a pelvic mass. Compared to OVA1, OVERA had improved sensitivity and specificity of 91% and 69%, respectively [114].

Combining transthyretin (TTR) with CA125, hemoglobin, apolipoprotein AI, and transferrin improved the detection of early-stage ovarian cancer compared to CA125 [115]. Another combination of CA125, HE4, leptin, osteopontin, and prolactin showed improved AUC compared to CA125 alone [116]. CA125, in combination with annexin A2 (ANXA2), showed 100% sensitivity and improved accuracy in distinguishing stage IA ovarian cancer from benign tumors [117]. The following two multivariate models not only performed better but also anticipated the diagnosis of ovarian cancer by 5 months to 2 years compared to CA125 alone. The first model used insulin-like growth factor-binding protein-2 (IGFBP-2), lecithin cholesterol acyltransferase (LCAT), and CA125. In contrast, the second one was based on a combination of CA125, HE4, arginase 2 (AGR2), phosphatidylethanolamine binding protein 4 (PEBP4), and chitinase-3-like protein 1 (CHI3L1) [118][119]. A combination of ROMA, HE4, CA125, the neutrophil-to-lymphocyte ratio (NLR), and lactate dehydrogenase (LD) with machine-learning algorithms could improve the prediction of ovarian cancer with AUC from 0.62 to 0.76. Both LD and NLR were correlated with survival in ovarian cancer patients [120]. In the European Prospective Investigation into Cancer and Nutrition cohort of patients and ovarian cancer-free women, nine possible markers were chosen from 92 preselected proteins. These markers included CA125, HE4, FOLR1, kallikrein-related peptidase 11 (KLK11), WNT1-inducible-signaling pathway protein 1 (WISP1), midline or neurite growth-promoting factor 2 (MDK), C-X-C motif chemokine ligand

13 (CXCL13), mesothelin (MSLN), and ADAM metallopeptidase domain 8 (ADAM8). These markers showed good discrimination between groups for a lag time of 0–9 months; however, none of the new markers improved the prediction when added to CA125 [121]. The panel of 26 proteins in plasma associated with the dysregulation of Hippo, Hippo-Merlin, Focal-Adhesion-PI3K/AKT/mTOR, PI3K/AKT, Ras, Ebola virus, and NOTCH signaling pathways was able to identify patients with HGSOC risk at a false discovery rate of <0.05 [122].

2.1.7. Urinary Tests

The evaluation of urinary biomarkers for the detection of ovarian cancer is a new and dynamic diagnostic possibility. Similar to serum-based studies, urine component searches are based on single or multiple markers. The marking of CA125 in urine samples gave a sensitivity of 89% and a specificity of 67% [123]. The subsequent urine marker tested was HE4, which provided a sensitivity of 51–89% and a specificity of 75–100%, depending on the study, and showed comparable accuracy to the serum testing [124][125][126]. The mesothelin urine assay showed that a more significant fraction of patients with both early-stage and advanced-stage ovarian cancer was detected with a urine assay (42% and 75%, respectively) compared to a serum assay (12% and 48%, respectively) [127]. Elevated levels of the anti-apoptotic protein Bcl-2 were present in urine from patients with ovarian cancer, independently from tumor stage, grade, and size, and differentiated between healthy and ovarian cancer patients with an AUC of 0.9 [128]. Urine high mobility group AT-hook 1 (HMGA1) protein was significantly elevated in serous epithelial ovarian cancer, and a higher AUC (0.88) was noted for urine HMGA1 than for serum CA125 [129]. The AUC of 0.68 for differentiating ovarian cancer from benign disease was pointed out for the urine minichromosome maintenance complex component 5 (MCM5) protein concentration [130].

Similarly to the serum, the multiple markers were also marked in urine. The combination of HE4, creatinine, carcinoembryonic antigen (CEA), and transthyretin (TTR) had 93.7% sensitivity and 70.6% specificity in predicting ovarian cancer compared to benign tumors [131]. The different combinations of the urine levels of two metalloproteinases (MMP-2 and MMP-9) and MMP-9/neutrophil gelatinase-associated lipocalin (NGAL) complex and age were evaluated in ovarian cancer compared to benign tumors. The best prediction with an AUC of 0.88 was obtained for MMP-2, MMP-9, and age [132]. Combining urinary eosinophil-derived neurotoxin (EDN) and osteopontin resulted in a sensitivity of 72% and specificity of 95% to distinguish ovarian cancer from normal controls and elevation in early-stage ovarian cancer [133]. MiR-30a-5p was shown to be up-regulated in HGSOC, while urinary miR-30a-5p was notably reduced following the surgical removal of ovarian tumors. The miR-30a-5p was concentrated within the exosomes from ovarian cancer or urine from HGSOC patients. MiR-30a-5p showed good discrimination between HGSOC patients and healthy controls with an AUC of 0.86 [134]. Several proteins, including LY6/PLAUR domain containing 1 (LYPD1), lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1), prothymosin alpha (PTMA), and secretoglobin family 1A member 1 (SCGB1A1) were confirmed to be increased in the urine of ovarian cancer patients compared to benign ovarian tumors, giving an AUC of 0.92 [135]. The combination of fibrinogen α fragment, collagen α 1 fragment, and fibrinogen β NT fragment showed an AUC of 0.88 for discrimination between ovarian cancer and benign ovarian tumors. The AUC was enhanced to 0.96 by an additional combination with CA125 [136].

2.1.8. Epigenetic Markers

Micro RNAs belonging to non-coding regulatory RNAs play an essential role in changing the expression of many target genes and intercellular communication. The levels of several miRNAs were investigated in serum, plasma, and circulating peripheral blood exosomes. The panel of eight miRNAs was both up-regulated (miR-21, miR29a, miR92, miR-93, and miR-126) and down-regulated (miR-99, miR-127, miR-155) in the serum of ovarian cancer patients compared to normal controls [137]. In the next studied panel of plasma 11 miRNAs, the up-regulation of miR-191-5p, miR-206, miR-34c-5p, miR-548a-3p, miR-320a, miR-574-3p, miR-590-5p, and miR-106b-5p and the down-regulation of miR-19a-3p, miR-30a-5p, miR-645, and miR-150-5p was noticed [138]. Similarly, serum expression of miR-145 and miR-133b was significantly decreased in ovarian cancer patients [139]. Moreover, the combined detection of miR-193-5p with CA125 and HE4 improved the accuracy of the diagnosis of ovarian cancer with an AUC of 0.996 [140]. MiR-200a, miR-200b, and miR-200c were significantly increased in the serum of HGSOC patients, with miR0-200b + miR-200c having the power to discriminate between ovarian cancer and healthy patients with an AUC of 0.78 [141]. A set of ten different miRNAs (miR-4687-3p, miR-939-5p, miR-5739, miR-211-3p, miR-1273g-3p, miR-3663-3p, miR-4726-5p, miR-4745-5p, miR-1268b, and miR-658) provided a prediction of ovarian cancer with an AUC of 0.97. However, every individual miRNA from the set was insufficient to discriminate cancer patients from healthy subjects when used alone [142]. The different sets of ten serum miRNAs enabled the identification of patients at risk of HGSOC. The set was constructed from miR-5100, miR-6800-5p, miR-1233-5p, miR-4532, miR-4783-3p, miR-4787-3p, miR-1228-5p, miR-1290, miR-3184-5p, and miR-320b and identified at-risk patients with an AUC of 1.00 [143]. A serum miRNA neural network of seven miRNAs (miR-29a-3p, miR-92a-3p, miR-200c-3p, miR- 320c, miR-335–5p, miR-450b-5p, and miR-1307–5 p) tested on an independent external sample of ovarian cancer showed a diagnostic AUC of 0.85. Moreover, the in situ hybridization of three miRNAs from the network

(mir-200c-3p, mir-335–5 p, and mir-92a-3p) showed a complete overlap between the serum miRNAs and the miRNAs expressed in serous tubal intraepithelial cancer (STIC) and early stage I HGSOC [144]. The combination of miR-320b and miR-141-3p alone or integrated with CA125 and HE4 showed the accurate discrimination capability of early-stage ovarian cancer and healthy controls (AUC 0.79 and 1.00, respectively) [145]. Finally, miR-204, CA125, and CA19.9 panels showed an AUC of 0.94, 1.00, and 0.99 for benign, early-stage, and advanced ovarian cancer tumors, respectively [146]. Besides miRNA, another group of non-coding RNA called circular RNA has been tested for diagnostic accuracy. Circ _0003972 and circ_0007288 were down-regulated in the plasma of ovarian cancer patients and acted as diagnostic markers with an AUC of 0.78 and an AUC of 0.92 when combined with CA125. Both circ_RNAs function as tumor suppressors by sponging oncogenic miRNAs [147]. Tumor-associated molecules could be transferred to platelets, which are then educated to induce the specific splicing of pre-messenger RNA. These are called tumor-educated platelets (TEPs) and were tested as diagnostic tools in differentiating between early-stage ovarian cancer and benign tumors with 80% accuracy [148]. The usefulness of TEPs is evaluated in clinical trials (ClinicalTrials.gov identifier: NCT04022863).

An exciting possibility comes from the study demonstrating the use of a DNA-methylation signature of cervical cells to identify both ovarian and endometrial cancer. The signature Women's Risk Identification for Ovarian Cancer Index (WID-OC index) enabled the identification of women with ovarian cancer with an AUC of 0.76. Interestingly, the WID-OC index was not connected with the presence of ovarian tumor DNA in cervical samples but rather with cervical epigenetic profiles originating from disturbed Müllerian duct differentiation [149]. Testing the DNA methylation according to meta-analysis showed better performance for the prediction of ovarian cancer than cell-free tumor DNA with an AUC of 0.93 [150]. The homeobox A9 (HOXA9) and hypermethylated cancer-1 (HIC1) promoter methylation in serum cell-free DNA samples showed an AUC of 0.95 in discrimination between ovarian cancer and healthy controls [151][152]. The plasma cell-free DNA (cfDNA) methylation pattern has been shown to have diagnostic potential. The detection model based on cfDNA methylation achieved a sensitivity of 95% and a specificity of 89%, outperforming the CA125 [153].

2.1.9. Autoantibodies (AAbs)

The most common mutation in HGSOC is the *TP53* mutation, which is present in over 95% of cancer cases [154]. The mutation is observed even in the early stages of HGSOC, and the lead time to the diagnosis using anti-TP53 serum AAbs is about 8–9 months, enabling the earlier prediction of cancer than with the use of CA125 or ROCA [155]. However, the anti-TP53 indicated minimal sensitivity as a single marker. Moreover, the presence of anti-TP53 AAbs is observed in only 40% of HGSOC patients and correlates with tumor burden. The combination of anti-TP53 AAbs with CA125 improved the accuracy of CA125 alone. The panel of three anti-tumor-associated antibodies, anti-G-protein alpha subunit Gs-α (GNAS), anti-TP53, and anti-nucleophosmin 1 (NMP1), could identify 51% of ovarian cancer patients negative for CA125 [156]. Paraneoplastic tripartite motif-containing protein 21 (TRIM21) AAbs in combination with anti-New York esophageal squamous cell carcinoma 1 (NY-ESO-1), anti-TP53, and anti-paired box 8 (PAX8, marker of the Fallopian tube secretory cell lineage) AAbs tested in the group of patients with increased risk of ovarian cancer (positive family history, Lynch syndrome, or *BRCA* germinal mutations) showed 46% sensitivity and 98% specificity. It could complement CA125 screening in this group of women [157]. Another panel of nine AAbs (anti-TP53, C-myc, protein kinase p90, ubiquitin-binding p62, alpha 2-HS glycoprotein AHSG, ubiquitously expressed 14-3-3zeta, Ras-related protein RalA, KH domain-containing protein over-expressed in cancer Koc, and tumor suppressor p16) showed an AUC of 0.91 and identified 79% of ovarian cancer patients presenting with normal CA125 levels [158]. Autoantibodies against leucine repeat death domain-containing protein (LRDD), stanniocalcin-1 (STC1), and forkhead box a1 (FOXA1) were over-expressed in ovarian cancer patients compared to normal controls and showed an AUC of 0.91, 0.88 and 0.82, respectively. The best discriminating combination was anti-LRDD + anti-FOXA1 AAbs, and the accuracy of this set improved when further combined with CA125 [159]. This promising combination was based on anti-BRCA1-associated RING domain 1 (BARD1) and CA125 levels. It showed high specificity and sensitivity (over 90%) for distinguishing ovarian cancer patients from healthy controls both for average-risk and high-risk women with hereditary breast and ovarian cancer syndrome (HBOC) [160]. Anti-PDZ and LIM domain-1 (PDLIM1) AAbs were able to differentiate both ovarian cancer patients from healthy controls (AUC 0.76) and ovarian benign tumors (AUC 0.76), as well as to identify 41% of early-stage and 39% of late-stage ovarian cancer, respectively. The anti-PDLIM1 AAbs could also find 15% of ovarian cancer patients negative on the base of CA125 marking alone. The combination with CA125 improved the AUC to 0.85 [161]. The panel of serum C-C motif chemokine ligand 18 (CCL18) and C-X-C motif chemokine ligand 1 (CXCL1) antigens combined with nucleic acid-binding protein (C1D), fragile X mental retardation syndrome-related protein 1 (FXR1), zinc finger protein 573 (ZNF573) and transmembrane 4 L six family member 1 (TM4SF1) AAbs showed an AUC of 0.99 for ovarian cancer diagnosis, an AUC of 0.95 for early-stage ovarian cancer, and an AUC of 0.75–0.96 for discriminating patients with ovarian cancer from patients with other malignancies [162]. The systematic review from Heidelberg [163] showed that the highest individual sensitivity was obtained with anti-Rho GDP-dissociation inhibitor (RhoGDI) and anti-tubulin alpha 1c (TUBA1C) AAbs but with concomitant low specificity. Better accuracy was obtained for anti-homeobox A7 (HOXA7) and anti-interleukin-8 (IL8)

AAbs for detecting moderately differentiated and early-stage tumors, respectively. The panel of eleven AAbs (intercellular adhesion molecule 3-ICAM3, cancer/testis antigen 2-CTAG2, transcription factor p53, serine/threonine/tyrosine interacting like 1-STYXL1, PVR cell adhesion molecule-PVR, proopiomelanocortin-POMC, Nudix hydrolase 11-NUDT11, tripartite motif containing 39-TRIM39, serine/threonine protein kinase Kist-UHMK1, kinase suppressor of ras 1-KSR1, and nuclear RNA export factor 3-NXF3) provided 45% sensitivity at 98% specificity for HGSOC. Generally, limited data suggest that AAbs could improve diagnostic discrimination when combined with CA125 and HE4 [163].

2.2. Assessment of Adnexal Mass-Prediction of Ovarian Cancer in Symptomatic Patients

2.2.1. IOTA-SR

The IOTA Simple Rules were devised by the International Ovarian Tumor Analysis (IOTA) group to predict malignancy of the recognized adnexal mass [164]. The model is based on the ultrasound estimation of B (benign) and M (malignant) features of the adnexal tumor, which helps to classify the patient into gynecologic oncology assessment. IOTA-SR predicts ovarian malignancy with a 92% sensitivity and 96% specificity and is more accurate than RMI or ROMA alone [165]. Therefore, the IOTA should be the first method to assess a pelvic mass, and its results should be confirmed using an expert ultrasound. The researchers believe the IOTA system could be used for screening in postmenopausal women.

2.2.2. Multivariate Assays

The Risk of Malignancy Index (RMI) combines TVUS, CA125 level, and menopausal status and expresses the result as a calculated product of these components. The cut-off value of 200 has better sensitivity and specificity than the CA125 level alone [166][167]. Using different values for TVUS and menopausal status, four RMI algorithms were created; however, their clinical efficacy illustrated by the AUC did not prove to differ between them [168].

The multivariate index Risk of Malignancy Algorithm (ROMA) has incorporated the CA125 serum level, HE4, and menopausal status [109]. Compared to other assays, ROMA showed satisfactory specificity (91%) and sensitivity (90%) in the diagnosis of ovarian cancer [169]. However, the ROMA index showed a lower specificity in postmenopausal compared to premenopausal women [170]. Compared to CA125, ROMA showed improved specificity, especially in premenopausal women, as well as higher sensitivity compared with the RMI [171].

2.3. Screening Trials

The problem of screening for ovarian cancer has not been solved so far, although several attempts at screening in the average-risk postmenopausal women population have been undertaken. The prevalence of pathology in the population determines the demands for effective screening, which, in the case of ovarian cancer, taking into account the incidence of 40 cases per 100,000 persons/year, means that the test accuracy required to achieve a 10% positive predictive value in the whole population demands a sensitivity of 60–100% and a specificity of 99.6–99.8% [172]. In postmenopausal women, the test should have a sensitivity more significant than 75% and a specificity greater than 99.6% to reach a positive predictive value of 10% for the detection of early cancer. That means that based on the screening test itself, it would be nine false positive cases per one case of true ovarian cancer, which is an acceptable result. Using a single test is insufficient to attain this screening efficacy level. Therefore, the solution is to use either multimodal testing or to combine it with serial testing [173]. Several attempts at screening in the average-risk postmenopausal women population have been undertaken. In the Kentucky Study, women over 50 with no risk and women over 25 from risk families were included and received annual transvaginal ultrasounds, followed by detailed diagnostic procedures in suspected cases. The 5-year survival rate for women with ovarian cancer detected by screening was 75% compared with 54% for unscreened women with ovarian cancer treated in the same way [174]. The Shizuoka Cohort Study of Ovarian Cancer Screening (SCSOCS) recruited asymptomatic postmenopausal women for annual transvaginal ultrasound and CA125 (intervention group n = 41,688 vs. control group n = 40,799). Eight more cancers were diagnosed outside the screening program. The proportion of early-stage ovarian cancer was higher in the screened group (63%) than in the control group (38%); however, this did not reach statistical significance [175]. Extended follow-up of the Prostate, Lung, Colorectal, and Ovarian (PLCO) Cancer Screening Trial indicated no mortality benefit from screening for ovarian cancer with annual CA125 and transvaginal ultrasound [176]. However, the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS) showed an overall mortality reduction of 20% in favor of multimodal screening (ROCA algorithm + transvaginal ultrasound in the group of patients with raised ROCA) [166]. In the subsequent trial, called the Normal Risk of Ovarian Cancer screening study (NROSS), postmenopausal women were tested using the ROCA algorithm and then selected for consecutive annual CA125 tests (low risk), repeat CA125 test in 3 months (intermediate risk), or transvaginal ultrasound and gynecologic oncologist consultation (high risk). The authors concluded that ROCA followed by transvaginal ultrasound demonstrated 99.9% specificity in a population of women at average risk for ovarian cancer [177]. Due to these discrepancies, screening

programs have yet to be implemented commonly in clinics, and the efficacy of such proceedings still needs confirmation in randomized trials. An interesting problem is the question of how early the increase in biomarkers precedes the diagnosis of ovarian cancer (pre-diagnostic efficacy). Generally, for CA125, the lead time (the time to diagnosis) is shorter than one year and, in most studies, does not overrun six months. It has a higher sensitivity for advanced cancer (FIGO III-IV). The same is true for other biomarkers (HE4, CA72.4); however, panels of biomarkers outperform single biomarkers like CA125, having a longer lead time [118][178].

2.4. Liquid Biopsy

2.4.1. Circulating Tumor Cells

Circulating tumor cells (CTCs) are the cells shed from the tumor, intravasated, and found in the peripheral blood. The isolation of CTCs is challenging due to the relative scarcity of tumor cells in all circulating cells, mainly of hematological lineage [179]. Most CTCs undergo both apoptosis and necrosis due to the hostile environment in the blood, including starvation, stress, and immunologically mediated elimination [180]. The most popular and approved system to isolate CTCs is a CellSearch detection system based on isolating cells with positive epithelial cell adhesion molecule (EpCAM) expression [181]. However, its efficacy is limited due to varied numbers of EpCAM(+) CTCs [182]. Novel approaches capable of omitting this limitation use CTC enrichment methods with multiple identification markers or the utilization of CTC's ability to invade the artificial matrix [183][184][185].

One of the first publications devoted to CTCs identified them in the blood of 12% of ovarian cancer patients, using an immuno-microbead that recognized an EpCAM, regularly expressed on ovarian cancer cells [186]. Another early paper confirmed the evidence of CTCs in the peripheral blood of 19% of ovarian cancer patients, especially with most malignant grade 3 tumors. The CTCs were identified using cytokeratins CK7, CK8 CK18, and CK20, transcription factor-2 (TFS-2), and epithelial growth factor receptor (EGFR) [187]. The study detecting CTCs identified as cells positive for EpCAM, CK 4, 5, 6, 8, 10, 13, and 18 showed CTC positivity in 10% of early-stage cancer patients and 73% of late-stage patients. Patients with advanced cancer showed higher numbers of peripheral blood CTCs [188]. In another study, CTCs were detected before surgery in 19% of patients expressing EpCAM (31%), MUC-1 (50%), HER-2 (31%), and CA 125 (50%), respectively [189]. In another study, CTCs were isolated on the base of EpCAM/CK positivity combined with physical cellular features like cell size and deformability in patients with benign and malignant ovarian tumors. A total of 56% of cases were positive for CTCs, including 100% early-stage and 68% advanced-stage cancer. However, 44% of patients with benign tumors also showed the presence of cells interpreted as CTCs. The CTC detection had a sensitivity of 77%, 100%, and 100% for benign vs. all-stage cancer, benign vs. stage I-II cancer, and benign vs. stage I cancer, respectively [190]. In the next study, the CTC count established by a microfluidic method differed between healthy and ovarian cancer patients. CTCs defined as Hoechst+, CK+, and CD45- cells showed counts of more than eight in 87% of ovarian cancer patients but in none of the healthy volunteers, and the total CTC counts were found to be significantly elevated in the ovarian cancer group (55 vs. 0.5) even though CTC-like cells were identified in low numbers in healthy controls [191]. The immunomagnetic targeting of combined mesenchymal N-cadherin and epithelial EpCAM-positive cells enriched the CTC population approximately three times more efficiently than targeting EpCAM alone. According to previous studies, in some blood samples of healthy individuals, the presence of cells expressing markers common to CTCs was also observed. Analysis showed that these "false positives" were identified as circulating endothelial cells (CECs) by vascular endothelial-cadherin co-staining and that their count could be highly variable in patients and healthy controls [192]. This is a reliable explanation for the identification of CTC-like cells in benign tumors and healthy individuals. The CTCs marked as CD45-/HE4+EpCAM+cytokeratin+vimentin+ cells were increased in ovarian cancer patients and showed positivity in 49% of patients with a sensitivity of 73%, higher than that for CA125 [193]. Comparison of the gene expression level in the group of CTC-negative and -positive peripheral blood samples confirmed a statistically significant difference for the expression of the Wilms tumor *WT1*, *EPCAM*, *MUC16*, *MUC1*, keratin *KRT7*, *KRT18*, and *KRT19* genes [194]. Another study of the genetic pattern of epithelial EpCAM+ CTCs showed the expression of *MUC1* and cytokeratin 19 (*CK19*) but also of genes associated with mesenchymal and more malignant features such as tissue inhibitor of metallopeptidase 1 (*TIMP1*), C-X-C chemokine receptor type 4 (*CXCR4*), and the stem markers *CD24* and *CD44* [195].

The evolution of isolation techniques improved the CTC detection rate to almost 90% of ovarian cancer patients and increased the number of CTCs correlated with the clinical stage of the tumor. Moreover, patients with early-stage disease had a CTC-positive rate of 93% compared to CA125, which was positive at merely 64% [196]. The combination of anti-EpCAM-moAbs and anti-FRα-moAbs showed a significantly increased positive rate of CTC detection in ovarian cancer patients compared with anti-EpCAM-moAbs alone [197]. Recently, the detection model of CTCs based on the expression of EpCAM, MUC1, and Wilms tumor protein WT1 showed significantly higher specificity than CA125 (92% vs. 82%),

especially in early-stage ovarian cancer (74% vs. 58%). The sensitivity of the detection, as mentioned in the above model, ranged up to 79.4% [198].

2.4.2. Cell-Free DNA/Circulating Tumor DNA

Cell-free DNA (cfDNA) is a DNA released from apoptotic or necrotic cells and contains a subpopulation of circulating tumor DNA (ctDNA) in the case of cancer. The ctDNA is a more achievable target than CTCs due to its relative abundance [199]. The amount of cfDNA varies in plasma in wide borders (3–93%) and depends on the cancer's presence and the tumor size [200]. The ctDNA fragments' length is usually shorter than cfDNA; therefore, searching for shorter fragments could enrich the sample in ctDNA [201]. Moreover, cfDNA Integrity (cfDI) indicates the ratio of long necrosis-derived DNA fragments to short apoptosis-derived fragments. Cancer patients have a higher cfDI compared to healthy controls and benign tumors [202]. The stability of cfDNA in the blood depends on the amount of DNA released and the clearance of DNA, which, in the case of cancer, is significantly decreased [203]. The estimated time of the half-life of cfDNA was around 4–30 min [204]. The ctDNA detection is based on several methods, including quantitative polymerase chain reaction (PCR), digital droplet PCR, next-generation sequencing (NGS), and whole-genome sequencing (WGS) [205]. Combining NGS with targeted error correction sequencing (TEC-Seq) or duplex sequencing can improve further detection accuracy [206][207].

The main challenge in ovarian cancer is to recognize tumors in their early stages when surgery is much easier and the prognosis is better than in the advanced stage. The early phenomenon in ovarian cancer is promoter methylation of suppressor genes, which could be used as a diagnostic tool [208][209]. The methylation of opioid-binding protein/cell adhesion molecules like *OPCML*, Runt-related transcription factor 3 (*RUNX3*), and tissue factor pathway inhibitor 2 (*TFPI2*) genes was studied in ovarian cancer patients using methylation-specific NGS. The methylation of *OPCML* showed a significant difference between early-stage ovarian cancer patients and healthy controls, even when CA125 did not differ between them. Ras-association domain family 2A (*RASSF2A*) is a suppressor gene whose down-regulated expression has been described in several malignancies. The epigenetic inactivation of *RASSF2A* through aberrant promoter methylation was found in 36% of ovarian cancer plasma samples [210]. The multiplex assay of aberrantly methylated seven genes (*RASSF1A*, *RUNX3*, *TFPI2*, *OPCML*, secreted frizzled-related protein 5-*SFRP5*, cadherin 1-*CDH1*, and APC regulator of WNT signaling pathway-*APC*) achieved a sensitivity of 85% and a specificity of 90% in early-stage ovarian cancer, which was surprisingly better than the result obtained for CA125 [211]. The following suppressor gene, the slit homolog 2 (*SLIT2*), was shown to be aberrantly methylated. Among the cases with hypermethylation in ovarian tissue, 93% of the case-matched serum DNA samples also showed hypermethylation, including cases of early-stage ovarian cancer [212].

References

1. Webb, P.M.; Jordan, S.J. Epidemiology of Epithelial Ovarian Cancer. Best Pract. Res. Clin. Obstet. Gynaecol. 2017, 41, 3–14.
2. Gong, T.-T.; Wu, Q.-J.; Vogtmann, E.; Lin, B.; Wang, Y.-L. Age at Menarche and Risk of Ovarian Cancer: A Meta-Analysis of Epidemiological Studies. Int. J. Cancer 2013, 132, 2894–2900.
3. Wentzensen, N.; Poole, E.M.; Trabert, B.; White, E.; Arslan, A.A.; Patel, A.V.; Setiawan, V.W.; Visvanathan, K.; Weiderpass, E.; Adami, H.-O.; et al. Ovarian Cancer Risk Factors by Histologic Subtype: An Analysis From the Ovarian Cancer Cohort Consortium. J. Clin. Oncol. 2016, 34, 2888–2898.
4. Collaborative Group on Epidemiological Studies of Ovarian Cancer. Ovarian Cancer and Oral Contraceptives: Collaborative Reanalysis of Data from 45 Epidemiological Studies Including 23 257 Women with Ovarian Cancer and 87 303 Controls. Lancet 2008, 371, 303–314.
5. Collaborative Group on Epidemiological Studies of Ovarian Cancer. Menopausal Hormone Use and Ovarian Cancer Risk: Individual Participant Meta-Analysis of 52 Epidemiological Studies. Lancet 2015, 385, 1835–1842.
6. Sieh, W.; Salvador, S.; McGuire, V.; Weber, R.P.; Terry, K.L.; Rossing, M.A.; Risch, H.; Wu, A.H.; Webb, P.M.; Moysich, K.; et al. Tubal Ligation and Risk of Ovarian Cancer Subtypes: A Pooled Analysis of Case-Control Studies. Int. J. Epidemiol. 2013, 42, 579–589.
7. Fucic, A.; Gamulin, M.; Ferencic, Z.; Katic, J.; Krayer Von Krauss, M.; Bartonova, A.; Merlo, D.F. Environmental Exposure to Xenoestrogens and Oestrogen Related Cancers: Reproductive System, Breast, Lung, Kidney, Pancreas, and Brain. Environ. Health 2012, 11, S8.
8. Ogura, K.; Irahara, M.; Kiyokawa, M.; Tezuka, M.; Matsuzaki, T.; Yasui, T.; Kamada, M.; Aono, T. Effects of Leptin on Secretion of LH and FSH from Primary Cultured Female Rat Pituitary Cells. Eur. J. Endocrinol. 2001, 144, 653–658.

9. Merritt, M.A.; Cramer, D.W.; Missmer, S.A.; Vitonis, A.F.; Titus, L.J.; Terry, K.L. Dietary Fat Intake and Risk of Epithelial Ovarian Cancer by Tumour Histology. Br. J. Cancer 2014, 110, 1392–1401.
10. Whiteman, D.C.; Webb, P.M.; Green, A.C.; Neale, R.E.; Fritschi, L.; Bain, C.J.; Parkin, D.M.; Wilson, L.F.; Olsen, C.M.; Nagle, C.M.; et al. Cancers in Australia in 2010 Attributable to Modifiable Factors: Summary and Conclusions. Aust. N. Z. J. Public Health 2015, 39, 477–484.
11. Cunningham, J.M.; Cicek, M.S.; Larson, N.B.; Davila, J.; Wang, C.; Larson, M.C.; Song, H.; Dicks, E.M.; Harrington, P.; Wick, M.; et al. Clinical Characteristics of Ovarian Cancer Classified by BRCA1, BRCA2 and RAD51C Status. Sci. Rep. 2014, 4, 4026.
12. Testa, U.; Petrucci, E.; Pasquini, L.; Castelli, G.; Pelosi, E. Ovarian Cancers: Genetic Abnormalities, Tumor Heterogeneity and Progression, Clonal Evolution and Cancer Stem Cells. Medicines 2018, 5, 16.
13. Ford, D.; Easton, D.F.; Stratton, M.; Narod, S.; Goldgar, D.; Devilee, P.; Bishop, D.T.; Weber, B.; Lenoir, G.; Chang-Claude, J.; et al. Genetic Heterogeneity and Penetrance Analysis of the BRCA1 and BRCA2 Genes in Breast Cancer Families. Am. J. Hum. Genet. 1998, 62, 676–689.
14. Ramus, S.J.; Song, H.; Dicks, E.; Tyrer, J.P.; Rosenthal, A.N.; Intermaggio, M.P.; Fraser, L.; Gentry-Maharaj, A.; Hayward, J.; Philpott, S.; et al. Germline Mutations in the BRIP1, BARD1, PALB2, and NBN Genes in Women With Ovarian Cancer. J. Natl. Cancer Inst. 2015, 107, djv214.
15. Song, H.; Dicks, E.; Ramus, S.J.; Tyrer, J.P.; Intermaggio, M.P.; Hayward, J.; Edlund, C.K.; Conti, D.; Harrington, P.; Fraser, L.; et al. Contribution of Germline Mutations in the RAD51B, RAD51C, and RAD51D Genes to Ovarian Cancer in the Population. J. Clin. Oncol. 2015, 33, 2901–2907.
16. Lisio, M.-A.; Fu, L.; Goyeneche, A.; Gao, Z.; Telleria, C. High-Grade Serous Ovarian Cancer: Basic Sciences, Clinical and Therapeutic Standpoints. Int. J. Mol. Sci. 2019, 20, 952.
17. Lyra, P.C.M.; Rangel, L.B.; Monteiro, A.N.A. Functional Landscape of Common Variants Associated with Susceptibility to Epithelial Ovarian Cancer. Curr. Epidemiol. Rep. 2020, 7, 49–57.
18. Lawrenson, K.; Iversen, E.S.; Tyrer, J.; Weber, R.P.; Concannon, P.; Hazelett, D.J.; Li, Q.; Marks, J.R.; Berchuck, A.; Lee, J.M.; et al. Common Variants at the CHEK2 Gene Locus and Risk of Epithelial Ovarian Cancer. Carcinogenesis 2015, 36, 1341–1353.
19. Graffeo, R.; Rana, H.Q.; Conforti, F.; Bonanni, B.; Cardoso, M.J.; Paluch-Shimon, S.; Pagani, O.; Goldhirsch, A.; Partridge, A.H.; Lambertini, M.; et al. Moderate Penetrance Genes Complicate Genetic Testing for Breast Cancer Diagnosis: ATM, CHEK2, BARD1 and RAD51D. Breast 2022, 65, 32–40.
20. Bonadona, V. Cancer Risks Associated With Germline Mutations in MLH1, MSH2, and MSH6 Genes in Lynch Syndrome. JAMA 2011, 305, 2304.
21. Engel, C.; Loeffler, M.; Steinke, V.; Rahner, N.; Holinski-Feder, E.; Dietmaier, W.; Schackert, H.K.; Goergens, H.; Von Knebel Doeberitz, M.; Goecke, T.O.; et al. Risks of Less Common Cancers in Proven Mutation Carriers With Lynch Syndrome. J. Clin. Oncol. 2012, 30, 4409–4415.
22. Ketabi, Z.; Bartuma, K.; Bernstein, I.; Malander, S.; Grönberg, H.; Björck, E.; Holck, S.; Nilbert, M. Ovarian Cancer Linked to Lynch Syndrome Typically Presents as Early-Onset, Non-Serous Epithelial Tumors. Gynecol. Oncol. 2011, 121, 462–465.
23. Watson, P.; Bützow, R.; Lynch, H.T.; Mecklin, J.-P.; Järvinen, H.J.; Vasen, H.F.A.; Madlensky, L.; Fidalgo, P.; Bernstein, I. The Clinical Features of Ovarian Cancer in Hereditary Nonpolyposis Colorectal Cancer. Gynecol. Oncol. 2001, 82, 223–228.
24. Sorrell, A.D.; Espenschied, C.R.; Culver, J.O.; Weitzel, J.N. Tumor Protein P53 (TP53) Testing and Li-Fraumeni Syndrome: Current Status of Clinical Applications and Future Directions. Mol. Diagn. Ther. 2013, 17, 31–47.
25. Olivier, M.; Goldgar, D.E.; Sodha, N.; Ohgaki, H.; Kleihues, P.; Hainaut, P.; Eeles, R.A. Li-Fraumeni and Related Syndromes: Correlation between Tumor Type, Family Structure, and TP53 Genotype. Cancer Res. 2003, 63, 6643–6650.
26. Schuijer, M.; Berns, E.M.J.J. TP53 and Ovarian Cancer. Hum. Mutat. 2003, 21, 285–291.
27. Chen, H.; Zhai, Z.; Xie, Q.; Lai, Y.; Chen, G. Correlation between SNPs of PIK3CA, ERBB2 3′UTR, and Their Interactions with Environmental Factors and the Risk of Epithelial Ovarian Cancer. J. Assist. Reprod. Genet. 2021, 38, 2631–2639.
28. Peedicayil, A.; Vierkant, R.A.; Hartmann, L.C.; Fridley, B.L.; Fredericksen, Z.S.; White, K.L.; Elliott, E.A.; Phelan, C.M.; Tsai, Y.-Y.; Berchuck, A.; et al. Risk of Ovarian Cancer and Inherited Variants in Relapse-Associated Genes. PLoS ONE 2010, 5, e8884.

29. Peedicayil, A.; Vierkant, R.A.; Shridhar, V.; Schildkraut, J.M.; Armasu, S.; Hartmann, L.C.; Fridley, B.L.; Cunningham, J.M.; Phelan, C.M.; Sellers, T.A.; et al. Polymorphisms in TCEAL7 and Risk of Epithelial Ovarian Cancer. Gynecol. Oncol. 2009, 114, 260–264.

30. Song, Z.-S.; Wu, Y.; Zhao, H.-G.; Liu, C.-X.; Cai, H.-Y.; Guo, B.-Z.; Xie, Y.; Shi, H.-R. Association between the Rs11614913 Variant of miRNA-196a-2 and the Risk of Epithelial Ovarian Cancer. Oncol. Lett. 2016, 11, 194–200.

31. Galaris, D.; Pantopoulos, K. Oxidative Stress and Iron Homeostasis: Mechanistic and Health Aspects. Crit. Rev. Clin. Lab. Sci. 2008, 45, 1–23.

32. Chornokur, G.; Lin, H.-Y.; Tyrer, J.P.; Lawrenson, K.; Dennis, J.; Amankwah, E.K.; Qu, X.; Tsai, Y.-Y.; Jim, H.S.L.; Chen, Z.; et al. Common Genetic Variation In Cellular Transport Genes and Epithelial Ovarian Cancer (EOC) Risk. PLoS ONE 2015, 10, e0128106.

33. Jogie-Brahim, S.; Feldman, D.; Oh, Y. Unraveling Insulin-Like Growth Factor Binding Protein-3 Actions in Human Disease. Endocr. Rev. 2009, 30, 417–437.

34. Terry, K.L.; Tworoger, S.S.; Gates, M.A.; Cramer, D.W.; Hankinson, S.E. Common Genetic Variation in IGF1, IGFBP1 and IGFBP3 and Ovarian Cancer Risk. Carcinogenesis 2009, 30, 2042–2046.

35. Mostowska, A.; Sajdak, S.; Pawlik, P.; Lianeri, M.; Jagodzinski, P.P. DNMT1, DNMT3A and DNMT3B Gene Variants in Relation to Ovarian Cancer Risk in the Polish Population. Mol. Biol. Rep. 2013, 40, 4893–4899.

36. White, K.L.; Schildkraut, J.M.; Palmieri, R.T.; Iversen, E.S.; Berchuck, A.; Vierkant, R.A.; Rider, D.N.; Charbonneau, B.; Cicek, M.S.; Sutphen, R.; et al. Ovarian Cancer Risk Associated with Inherited Inflammation-Related Variants. Cancer Res. 2012, 72, 1064–1069.

37. Li, Z.; Block, M.S.; Vierkant, R.A.; Fogarty, Z.C.; Winham, S.J.; Visscher, D.W.; Kalli, K.R.; Wang, C.; Goode, E.L. The Inflammatory Microenvironment in Epithelial Ovarian Cancer: A Role for TLR4 and MyD88 and Related Proteins. Tumor Biol. 2016, 37, 13279–13286.

38. Zhang, Z.; Zhao, H.; Chu, C.; Fu, X.; Liu, Y.; Wang, L.; Wei, R.; Xu, K.; Li, L.; Li, X. The Emerging Roles of TLR and cGAS Signaling in Tumorigenesis and Progression of Ovarian Cancer. Front. Pharmacol. 2022, 13, 1072670.

39. Zhu, Y.; Huang, J.-M.; Zhang, G.-N.; Zha, X.; Deng, B.-F. Prognostic Significance of MyD88 Expression by Human Epithelial Ovarian Carcinoma Cells. J. Transl. Med. 2012, 10, 77.

40. Kania, K.D.; Haręża, D.; Wilczyński, J.R.; Wilczyński, M.; Jarych, D.; Malinowski, A.; Paradowska, E. The Toll-like Receptor 4 Polymorphism Asp299Gly Is Associated with an Increased Risk of Ovarian Cancer. Cells 2022, 11, 3137.

41. Ghazy, A.A.; El-Etreby, N.M. Relevance of HLA-DP/DQ and ICAM-1 SNPs among Ovarian Cancer Patients. Front. Immunol. 2016, 7, 202.

42. Dareng, E.O.; Tyrer, J.P.; Barnes, D.R.; Jones, M.R.; Yang, X.; Aben, K.K.H.; Adank, M.A.; Agata, S.; Andrulis, I.L.; Anton-Culver, H.; et al. Polygenic Risk Modeling for Prediction of Epithelial Ovarian Cancer Risk. Eur. J. Hum. Genet. 2022, 30, 349–362.

43. Parkin, D.M. The Global Health Burden of Infection-Associated Cancers in the Year 2002. Int. J. Cancer 2006, 118, 3030–3044.

44. Pathak, S.; Wilczyński, J.R.; Paradowska, E. Factors in Oncogenesis: Viral Infections in Ovarian Cancer. Cancers 2020, 12, 561.

45. Lai, C.-H.; Hsueh, S.; Lin, C.-Y.; Huang, M.-Y.; You, G.-B.; Chang, H.-C.; Pao, C.C. Human Papillomavirus in Benign and Malignant Ovarian and Endometrial Tissues. Int. J. Gynecol. Pathol. 1992, 11, 210–215.

46. Atalay, F.; Taskiran, C.; Taner, M.Z.; Pak, I.; Or, M.; Tuncer, S. Detection of Human Papillomavirus DNA and Genotyping in Patients with Epithelial Ovarian Carcinoma. J. Obs. Gynaecol. Res. 2007, 33, 823–828.

47. Shanmughapriya, S.; SenthilKumar, G.; Vinodhini, K.; Das, B.C.; Vasanthi, N.; Natarajaseenivasan, K. Viral and Bacterial Aetiologies of Epithelial Ovarian Cancer. Eur. J. Clin. Microbiol. Infect. Dis. 2012, 31, 2311–2317.

48. Malisic, E.; Jankovic, R.; Jakovljevic, K. Detection and Genotyping of Human Papillomaviruses and Their Role in the Development of Ovarian Carcinomas. Arch. Gynecol. Obs. 2012, 286, 723–728.

49. Carlson, J.W.; Rådestad, A.F.; Söderberg-Naucler, C.; Rahbar, A. Human Cytomegalovirus in High Grade Serous Ovarian Cancer Possible Implications for Patients Survival. Medicine 2018, 97, e9685.

50. Paradowska, E.; Jabłońska, A.; Studzińska, M.; Wilczyński, M.; Wilczyński, J.R. Detection and Genotyping of CMV and HPV in Tumors and Fallopian Tubes from Epithelial Ovarian Cancer Patients. Sci. Rep. 2019, 9, 19935.

51. Bilyk, O.O.; Pande, N.T.; Pejovic, T.; Buchinska, L.G. The Frequency of Human Papilloma Virus Types 16, 18 in Upper Genital Tract of Women at High Risk of Developing Ovarian Cancer. Exp. Oncol. 2014, 36, 121–124.

52. Bernard, X.; Robinson, P.; Nominé, Y.; Masson, M.; Charbonnier, S.; Ramirez-Ramos, J.R.; Deryckere, F.; Travé, G.; Orfanoudakis, G. Proteasomal Degradation of P53 by Human Papillomavirus E6 Oncoprotein Relies on the Structural Integrity of P53 Core Domain. PLoS ONE 2011, 6, e25981.

53. Banerjee, S.; Tian, T.; Wei, Z.; Shih, N.; Feldman, M.D.; Alwine, J.C.; Coukos, G.; Robertson, E.S. The Ovarian Cancer Oncobiome. Oncotarget 2017, 8, 36225–36245.

54. Cinatl, J.; Vogel, J.-U.; Kotchetkov, R.; Wilhelm Doerr, H. Oncomodulatory Signals by Regulatory Proteins Encoded by Human Cytomegalovirus: A Novel Role for Viral Infection in Tumor Progression. FEMS Microbiol. Rev. 2004, 28, 59–77.

55. Caposio, P.; Orloff, S.L.; Streblow, D.N. The Role of Cytomegalovirus in Angiogenesis. Virus Res. 2011, 157, 204–211.

56. Yurochko, A.D.; Kowalik, T.F.; Huong, S.M.; Huang, E.S. Human Cytomegalovirus Upregulates NF-Kappa B Activity by Transactivating the NF-Kappa B P105/P50 and P65 Promoters. J. Virol. 1995, 69, 5391–5400.

57. Oberstein, A.; Shenk, T. Cellular Responses to Human Cytomegalovirus Infection: Induction of a Mesenchymal-to-Epithelial Transition (MET) Phenotype. Proc. Natl. Acad. Sci. USA 2017, 114, E8244–E8253.

58. Zhao, J.; Liang, Q.; Cheung, K.-F.; Kang, W.; Lung, R.W.M.; Tong, J.H.M.; To, K.F.; Sung, J.J.Y.; Yu, J. Genome-Wide Identification of Epstein-Barr Virus-Driven Promoter Methylation Profiles of Human Genes in Gastric Cancer Cells: EBV-Driven Methylation in Gastric Cancer. Cancer 2013, 119, 304–312.

59. Pandya, D.; Mariani, M.; McHugh, M.; Andreoli, M.; Sieber, S.; He, S.; Dowell-Martino, C.; Fiedler, P.; Scambia, G.; Ferlini, C. Herpes Virus MicroRNA Expression and Significance in Serous Ovarian Cancer. PLoS ONE 2014, 9, e114750.

60. Khoury, J.D.; Tannir, N.M.; Williams, M.D.; Chen, Y.; Yao, H.; Zhang, J.; Thompson, E.J.; the TCGA Network; Meric-Bernstam, F.; Medeiros, L.J.; et al. Landscape of DNA Virus Associations across Human Malignant Cancers: Analysis of 3,775 Cases Using RNA-Seq. J. Virol. 2013, 87, 8916–8926.

61. Sipos, A.; Ujlaki, G.; Mikó, E.; Maka, E.; Szabó, J.; Uray, K.; Krasznai, Z.; Bai, P. The Role of the Microbiome in Ovarian Cancer: Mechanistic Insights into Oncobiosis and to Bacterial Metabolite Signaling. Mol. Med. 2021, 27, 33.

62. Miao, R.; Badger, T.C.; Groesch, K.; Diaz-Sylvester, P.L.; Wilson, T.; Ghareeb, A.; Martin, J.A.; Cregger, M.; Welge, M.; Bushell, C.; et al. Assessment of Peritoneal Microbial Features and Tumor Marker Levels as Potential Diagnostic Tools for Ovarian Cancer. PLoS ONE 2020, 15, e0227707.

63. Poore, G.D.; Kopylova, E.; Zhu, Q.; Carpenter, C.; Fraraccio, S.; Wandro, S.; Kosciolek, T.; Janssen, S.; Metcalf, J.; Song, S.J.; et al. Microbiome Analyses of Blood and Tissues Suggest Cancer Diagnostic Approach. Nature 2020, 579, 567–574.

64. Wang, Q.; Zhao, L.; Han, L.; Fu, G.; Tuo, X.; Ma, S.; Li, Q.; Wang, Y.; Liang, D.; Tang, M.; et al. The Differential Distribution of Bacteria between Cancerous and Noncancerous Ovarian Tissues in Situ. J. Ovarian Res. 2020, 13, 8.

65. Rasmussen, C.B.; Kjaer, S.K.; Albieri, V.; Bandera, E.V.; Doherty, J.A.; Høgdall, E.; Webb, P.M.; Jordan, S.J.; Rossing, M.A.; Wicklund, K.G.; et al. Pelvic Inflammatory Disease and the Risk of Ovarian Cancer and Borderline Ovarian Tumors: A Pooled Analysis of 13 Case-Control Studies. Am. J. Epidemiol. 2017, 185, 8–20.

66. Rasmussen, C.B.; Jensen, A.; Albieri, V.; Andersen, K.K.; Kjaer, S.K. Is Pelvic Inflammatory Disease a Risk Factor for Ovarian Cancer? Cancer Epidemiol. Biomark. Prev. 2017, 26, 104–109.

67. Lin, H.-W.; Tu, Y.-Y.; Lin, S.Y.; Su, W.-J.; Lin, W.L.; Lin, W.Z.; Wu, S.-C.; Lai, Y.-L. Risk of Ovarian Cancer in Women with Pelvic Inflammatory Disease: A Population-Based Study. Lancet Oncol. 2011, 12, 900–904.

68. Brunham, R.C.; Gottlieb, S.L.; Paavonen, J. Pelvic Inflammatory Disease. N. Engl. J. Med. 2015, 372, 2039–2048.

69. Jonsson, S.; Oda, H.; Lundin, E.; Olsson, J.; Idahl, A. Chlamydia Trachomatis, Chlamydial Heat Shock Protein 60 and Anti-Chlamydial Antibodies in Women with Epithelial Ovarian Tumors. Transl. Oncol. 2018, 11, 546–551.

70. Trabert, B.; Waterboer, T.; Idahl, A.; Brenner, N.; Brinton, L.A.; Butt, J.; Coburn, S.B.; Hartge, P.; Hufnagel, K.; Inturrisi, F.; et al. Antibodies Against Chlamydia trachomatis and Ovarian Cancer Risk in Two Independent Populations. J. Natl. Cancer Inst. 2019, 111, 129–136.

71. Gulve, N.; Prusty, B.K.; Rudel, T. Chlamydia trachomatis Impairs Host Base Excision Repair by Downregulating Polymerase β. Cell. Microbiol. 2019, 21, e12986.

72. Mi, Y.; Gurumurthy, R.K.; Zadora, P.K.; Meyer, T.F.; Chumduri, C. Chlamydia trachomatis Inhibits Homologous Recombination Repair of DNA Breaks by Interfering with PP2A Signaling. mBio 2018, 9, e01465-18.

73. Zadora, P.K.; Chumduri, C.; Imami, K.; Berger, H.; Mi, Y.; Selbach, M.; Meyer, T.F.; Gurumurthy, R.K. Integrated Phosphoproteome and Transcriptome Analysis Reveals Chlamydia-Induced Epithelial-to-Mesenchymal Transition in Host Cells. Cell Rep. 2019, 26, 1286–1302.e8.

74. Kessler, M.; Hoffmann, K.; Fritsche, K.; Brinkmann, V.; Mollenkopf, H.-J.; Thieck, O.; Teixeira Da Costa, A.R.; Braicu, E.I.; Sehouli, J.; Mangler, M.; et al. Chronic Chlamydia Infection in Human Organoids Increases Stemness and Promotes Age-Dependent CpG Methylation. Nat. Commun. 2019, 10, 1194.

75. Morikawa, A.; Kawabata, A.; Shirahige, K.; Akiyama, T.; Okamoto, A.; Sutani, T. Altered Cervicovaginal Microbiota in Premenopausal Ovarian Cancer Patients. Gene 2022, 811, 146083.

76. Grice, E.A.; Segre, J.A. The Skin Microbiome. Nat. Rev. Microbiol. 2011, 9, 244–253.

77. Nené, N.R.; Reisel, D.; Leimbach, A.; Franchi, D.; Jones, A.; Evans, I.; Knapp, S.; Ryan, A.; Ghazali, S.; Timms, J.F.; et al. Association between the Cervicovaginal Microbiome, BRCA1 Mutation Status, and Risk of Ovarian Cancer: A Case-Control Study. Lancet Oncol. 2019, 20, 1171–1182.

78. Zhou, B.; Sun, C.; Huang, J.; Xia, M.; Guo, E.; Li, N.; Lu, H.; Shan, W.; Wu, Y.; Li, Y.; et al. The Biodiversity Composition of Microbiome in Ovarian Carcinoma Patients. Sci. Rep. 2019, 9, 1691.

79. Pelzer, E.S.; Allan, J.A.; Waterhouse, M.A.; Ross, T.; Beagley, K.W.; Knox, C.L. Microorganisms within Human Follicular Fluid: Effects on IVF. PLoS ONE 2013, 8, e59062.

80. Verstraelen, H.; Vilchez-Vargas, R.; Desimpel, F.; Jauregui, R.; Vankeirsbilck, N.; Weyers, S.; Verhelst, R.; De Sutter, P.; Pieper, D.H.; Van De Wiele, T. Characterisation of the Human Uterine Microbiome in Non-Pregnant Women through Deep Sequencing of the V1-2 Region of the 16S rRNA Gene. PeerJ 2016, 4, e1602.

81. Brewster, W.R.; Burkett, W.C.; Ko, E.M.; Bae-Jump, V.; Nicole McCoy, A.; Keku, T.O. An Evaluation of the Microbiota of the Upper Reproductive Tract of Women with and without Epithelial Ovarian Cancer. Gynecol. Oncol. Rep. 2022, 42, 101017.

82. Giampaolino, P.; Foreste, V.; Di Filippo, C.; Gallo, A.; Mercorio, A.; Serafino, P.; Improda, F.P.; Verrazzo, P.; Zara, G.; Buonfantino, C.; et al. Microbiome and PCOS: State-of-Art and Future Aspects. Int. J. Mol. Sci. 2021, 22, 2048.

83. Salliss, M.E.; Farland, L.V.; Mahnert, N.D.; Herbst-Kralovetz, M.M. The Role of Gut and Genital Microbiota and the Estrobolome in Endometriosis, Infertility and Chronic Pelvic Pain. Hum. Reprod. Update 2021, 28, 92–131.

84. Yang, J.; Tan, Q.; Fu, Q.; Zhou, Y.; Hu, Y.; Tang, S.; Zhou, Y.; Zhang, J.; Qiu, J.; Lv, Q. Gastrointestinal Microbiome and Breast Cancer: Correlations, Mechanisms and Potential Clinical Implications. Breast Cancer 2017, 24, 220–228.

85. Baker, J.M.; Al-Nakkash, L.; Herbst-Kralovetz, M.M. Estrogen–Gut Microbiome Axis: Physiological and Clinical Implications. Maturitas 2017, 103, 45–53.

86. Blaut, M.; Clavel, T. Metabolic Diversity of the Intestinal Microbiota: Implications for Health and Disease1. J. Nutr. 2007, 137, 751S–755S.

87. Felder, M.; Kapur, A.; Gonzalez-Bosquet, J.; Horibata, S.; Heintz, J.; Albrecht, R.; Fass, L.; Kaur, J.; Hu, K.; Shojaei, H.; et al. MUC16 (CA125): Tumor Biomarker to Cancer Therapy, a Work in Progress. Mol. Cancer 2014, 13, 129.

88. Sturgeon, C.M.; Duffy, M.J.; Walker, G. The National Institute for Health and Clinical Excellence (NICE) Guidelines for Early Detection of Ovarian Cancer: The Pivotal Role of the Clinical Laboratory. Ann. Clin. Biochem. 2011, 48, 295–299.

89. Høgdall, E.V.S.; Christensen, L.; Kjaer, S.K.; Blaakaer, J.; Kjærbye-Thygesen, A.; Gayther, S.; Jacobs, I.J.; Høgdall, C.K. CA125 Expression Pattern, Prognosis and Correlation with Serum CA125 in Ovarian Tumor Patients. Gynecol. Oncol. 2007, 104, 508–515.

90. Funston, G.; Mounce, L.T.; Price, S.; Rous, B.; Crosbie, E.J.; Hamilton, W.; Walter, F.M. CA125 Test Result, Test-to-Diagnosis Interval, and Stage in Ovarian Cancer at Diagnosis: A Retrospective Cohort Study Using Electronic Health Records. Br. J. Gen. Pract. 2021, 71, e465–e472.

91. Gu, Z.; He, Y.; Zhang, Y.; Chen, M.; Song, K.; Huang, Y.; Li, Q.; Di, W. Postprandial Increase in Serum CA125 as a Surrogate Biomarker for Early Diagnosis of Ovarian Cancer. J. Transl. Med. 2018, 16, 114.

92. Chen, Z.; Liang, Q.; Zeng, H.; Zhao, Q.; Guo, Z.; Zhong, R.; Xie, M.; Cai, X.; Su, J.; He, Z.; et al. Exosomal CA125 as A Promising Biomarker for Ovarian Cancer Diagnosis. J. Cancer 2020, 11, 6445–6453.

93. Wang, Y.-S.; Ren, S.-F.; Jiang, W.; Lu, J.-Q.; Zhang, X.-Y.; Li, X.-P.; Cao, R.; Xu, C.-J. CA125-Tn ELISA Assay Improves Specificity of Pre-Operative Diagnosis of Ovarian Cancer among Patients with Elevated Serum CA125 Levels. Ann. Transl. Med. 2021, 9, 788.

94. Salminen, L.; Nadeem, N.; Rolfsen, A.L.; Dørum, A.; Laajala, T.D.; Grènman, S.; Hietanen, S.; Heinosalo, T.; Perheentupa, A.; Poutanen, M.; et al. Exploratory Analysis of CA125-MGL and –STn Glycoforms in the Differential Diagnostics of Pelvic Masses. J. Appl. Lab. Med. 2020, 5, 263–272.

95. Kim, B.; Park, Y.; Kim, B.; Ahn, H.J.; Lee, K.-A.; Chung, J.E.; Han, S.W. Diagnostic Performance of CA 125, HE4, and Risk of Ovarian Malignancy Algorithm for Ovarian Cancer. J. Clin. Lab. Anal. 2019, 33, e22624.

96. Li, J.; Dowdy, S.; Tipton, T.; Podratz, K.; Lu, W.-G.; Xie, X.; Jiang, S.-W. HE4 as a Biomarker for Ovarian and Endometrial Cancer Management. Expert. Rev. Mol. Diagn. 2009, 9, 555–566.

97. Huang, J.; Chen, J.; Huang, Q. Diagnostic Value of HE4 in Ovarian Cancer: A Meta-Analysis. Eur. J. Obstet. Gynecol. Reprod. Biol. 2018, 231, 35–42.

98. Hamed, E.O.; Ahmed, H.; Sedeek, O.B.; Mohammed, A.M.; Abd-Alla, A.A.; Ghaffar, H.M.A. Significance of HE4 Estimation in Comparison with CA125 in Diagnosis of Ovarian Cancer and Assessment of Treatment Response. Diagn. Pathol. 2013, 8, 11.

99. Scaletta, G.; Plotti, F.; Luvero, D.; Capriglione, S.; Montera, R.; Miranda, A.; Lopez, S.; Terranova, C.; De Cicco Nardone, C.; Angioli, R. The Role of Novel Biomarker HE4 in the Diagnosis, Prognosis and Follow-up of Ovarian Cancer: A Systematic Review. Expert. Rev. Anticancer. Ther. 2017, 17, 827–839.

100. Lycke, M.; Ulfenborg, B.; Malchau Lauesgaard, J.; Kristjansdottir, B.; Sundfeldt, K. Consideration Should Be given to Smoking, Endometriosis, Renal Function (eGFR) and Age When Interpreting CA125 and HE4 in Ovarian Tumor Diagnostics. Clin. Chem. Lab. Med. (CCLM) 2021, 59, 1954–1962.

101. Anastasi, E.; Manganaro, L.; Granato, T.; Benedetti Panici, P.; Frati, L.; Porpora, M.G. Is CA72-4 a Useful Biomarker in Differential Diagnosis between Ovarian Endometrioma and Epithelial Ovarian Cancer? Dis. Markers 2013, 35, 331–335.

102. Shin, K.-H.; Kim, H.-H.; Kwon, B.S.; Suh, D.S.; Joo, J.K.; Kim, K.H. Clinical Usefulness of Cancer Antigen (CA) 125, Human Epididymis 4, and CA72-4 Levels and Risk of Ovarian Malignancy Algorithm Values for Diagnosing Ovarian Tumors in Korean Patients with and without Endometriosis. Ann. Lab. Med. 2020, 40, 40–47.

103. Negishi, Y.; Iwabuchi, H.; Sakunaga, H.; Sakamoto, M.; Okabe, K.; Sato, H.; Asano, G. Serum and Tissue Measurements of CA72-4 in Ovarian Cancer Patients. Gynecol. Oncol. 1993, 48, 148–154.

104. Parker, N.; Turk, M.J.; Westrick, E.; Lewis, J.D.; Low, P.S.; Leamon, C.P. Folate Receptor Expression in Carcinomas and Normal Tissues Determined by a Quantitative Radioligand Binding Assay. Anal. Biochem. 2005, 338, 284–293.

105. AOCS Study Group; Köbel, M.; Madore, J.; Ramus, S.J.; Clarke, B.A.; Pharoah, P.D.P.; Deen, S.; Bowtell, D.D.; Odunsi, K.; Menon, U.; et al. Evidence for a Time-Dependent Association between FOLR1 Expression and Survival from Ovarian Carcinoma: Implications for Clinical Testing. An Ovarian Tumour Tissue Analysis Consortium Study. Br. J. Cancer 2014, 111, 2297–2307.

106. Leung, F.; Dimitromanolakis, A.; Kobayashi, H.; Diamandis, E.P.; Kulasingam, V. Folate-Receptor 1 (FOLR1) Protein Is Elevated in the Serum of Ovarian Cancer Patients. Clin. Biochem. 2013, 46, 1462–1468.

107. Kurosaki, A.; Hasegawa, K.; Kato, T.; Abe, K.; Hanaoka, T.; Miyara, A.; O'Shannessy, D.J.; Somers, E.B.; Yasuda, M.; Sekino, T.; et al. Serum Folate Receptor Alpha as a Biomarker for Ovarian Cancer: Implications for Diagnosis, Prognosis and Predicting Its Local Tumor Expression: Serum FRA as a Biomarker for Ovarian Cancer. Int. J. Cancer 2016, 138, 1994–2002.

108. Zheng, X.; Chen, S.; Li, L.; Liu, X.; Liu, X.; Dai, S.; Zhang, P.; Lu, H.; Lin, Z.; Yu, Y.; et al. Evaluation of HE4 and TTR for Diagnosis of Ovarian Cancer: Comparison with CA-125. J. Gynecol. Obstet. Hum. Reprod. 2018, 47, 227–230.

109. Skates, S.J. Ovarian Cancer Screening: Development of the Risk of Ovarian Cancer Algorithm (ROCA) and ROCA Screening Trials. Int. J. Gynecol. Cancer 2012, 22, S24–S26.

110. Menon, U.; Gentry-Maharaj, A.; Burnell, M.; Singh, N.; Ryan, A.; Karpinskyj, C.; Carlino, G.; Taylor, J.; Massingham, S.K.; Raikou, M.; et al. 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 2021, 397, 2182–2193.

111. Zhu, C.; Zhang, N.; Zhong, A.; Xiao, K.; Lu, R.; Guo, L. A Combined Strategy of TK1, HE4 and CA125 Shows Better Diagnostic Performance than Risk of Ovarian Malignancy Algorithm (ROMA) in Ovarian Carcinoma. Clin. Chim. Acta 2022, 524, 43–50.

112. Bristow, R.E.; Smith, A.; Zhang, Z.; Chan, D.W.; Crutcher, G.; Fung, E.T.; Munroe, D.G. Ovarian Malignancy Risk Stratification of the Adnexal Mass Using a Multivariate Index Assay. Gynecol. Oncol. 2013, 128, 252–259.

113. Dunton, C.J.; Hutchcraft, M.L.; Bullock, R.G.; Northrop, L.E.; Ueland, F.R. Salvaging Detection of Early-Stage Ovarian Malignancies When CA125 Is Not Informative. Diagnostics 2021, 11, 1440.

114. Coleman, R.L.; Herzog, T.J.; Chan, D.W.; Munroe, D.G.; Pappas, T.C.; Smith, A.; Zhang, Z.; Wolf, J. Validation of a Second-Generation Multivariate Index Assay for Malignancy Risk of Adnexal Masses. Am. J. Obstet. Gynecol. 2016, 215, 82.e1–82.e11.

115. Kozak, K.R.; Su, F.; Whitelegge, J.P.; Faull, K.; Reddy, S.; Farias-Eisner, R. Characterization of Serum Biomarkers for Detection of Early Stage Ovarian Cancer. Proteomics 2005, 5, 4589–4596.

116. Hasenburg, A.; Eichkorn, D.; Vosshagen, F.; Obermayr, E.; Geroldinger, A.; Zeillinger, R.; Bossart, M. Biomarker-Based Early Detection of Epithelial Ovarian Cancer Based on a Five-Protein Signature in Patient's Plasma—A Prospective Trial. BMC Cancer 2021, 21, 1037.

117. Lokman, N.A.; Ricciardelli, C.; Stephens, A.N.; Jobling, T.W.; Hoffmann, P.; Oehler, M.K. Diagnostic Value of Plasma Annexin A2 in Early-Stage High-Grade Serous Ovarian Cancer. Diagnostics 2021, 11, 69.

118. Russell, M.R.; Graham, C.; D'Amato, A.; Gentry-Maharaj, A.; Ryan, A.; Kalsi, J.K.; Ainley, C.; Whetton, A.D.; Menon, U.; Jacobs, I.; et al. A Combined Biomarker Panel Shows Improved Sensitivity for the Early Detection of Ovarian Cancer Allowing the Identification of the Most Aggressive Type II Tumours. Br. J. Cancer 2017, 117, 666–674.

119. Whitwell, H.J.; Worthington, J.; Blyuss, O.; Gentry-Maharaj, A.; Ryan, A.; Gunu, R.; Kalsi, J.; Menon, U.; Jacobs, I.; Zaikin, A.; et al. Improved Early Detection of Ovarian Cancer Using Longitudinal Multimarker Models. Br. J. Cancer 2020, 122, 847–856.

120. Jeong, S.; Son, D.-S.; Cho, M.; Lee, N.; Song, W.; Shin, S.; Park, S.-H.; Lee, D.J.; Park, M.-J. Evaluation of Combined Cancer Markers With Lactate Dehydrogenase and Application of Machine Learning Algorithms for Differentiating Benign Disease From Malignant Ovarian Cancer. Cancer Control 2021, 28, 107327482110334.

121. Mukama, T.; Fortner, R.T.; Katzke, V.; Hynes, L.C.; Petrera, A.; Hauck, S.M.; Johnson, T.; Schulze, M.; Schiborn, C.; Rostgaard-Hansen, A.L.; et al. Prospective Evaluation of 92 Serum Protein Biomarkers for Early Detection of Ovarian Cancer. Br. J. Cancer 2022, 126, 1301–1309.

122. Considine, D.P.C.; Jia, G.; Shu, X.; Schildkraut, J.M.; Pharoah, P.D.P.; Zheng, W.; Kar, S.P. Genetically Predicted Circulating Protein Biomarkers and Ovarian Cancer Risk. Gynecol. Oncol. 2021, 160, 506–513.

123. Tay, S.K.; Chua, E.K. Correlation of Serum, Urinary and Salivary CA 125 Levels in Patients with Adnexal Masses. Ann. Acad. Med. Singap. 1994, 23, 311–314.

124. Macuks, R.; Baidekalna, I.; Donina, S. Urinary Concentrations of Human Epididymis Secretory Protein 4 (He4) in The Diagnosis of Ovarian Cancer: A Case-Control Study. Asian Pac. J. Cancer Prev. 2012, 13, 4695–4698.

125. Liao, J.B.; Yip, Y.Y.; Swisher, E.M.; Agnew, K.; Hellstrom, K.E.; Hellstrom, I. Detection of the HE4 Protein in Urine as a Biomarker for Ovarian Neoplasms: Clinical Correlates. Gynecol. Oncol. 2015, 137, 430–435.

126. Giamougiannis, P.; Silva, R.V.O.; Freitas, D.L.D.; Lima, K.M.G.; Anagnostopoulos, A.; Angelopoulos, G.; Naik, R.; Wood, N.J.; Martin-Hirsch, P.L.; Martin, F.L. Raman Spectroscopy of Blood and Urine Liquid Biopsies for Ovarian Cancer Diagnosis: Identification of Chemotherapy Effects. J. Biophotonics 2021, 14, e202100195.

127. Badgwell, D.; Lu, Z.; Cole, L.; Fritsche, H.; Atkinson, E.N.; Somers, E.; Allard, J.; Moore, R.G.; Lu, K.H.; Bast, R.C. Urinary Mesothelin Provides Greater Sensitivity for Early Stage Ovarian Cancer than Serum Mesothelin, Urinary hCG Free Beta Subunit and Urinary hCG Beta Core Fragment. Gynecol. Oncol. 2007, 106, 490–497.

128. Anderson, N.S.; Bermudez, Y.; Badgwell, D.; Chen, R.; Nicosia, S.V.; Bast, R.C.; Kruk, P.A. Urinary Levels of Bcl-2 Are Elevated in Ovarian Cancer Patients. Gynecol. Oncol. 2009, 112, 60–67.

129. Zhou, J.; Xie, M.; He, H.; Shi, Y.; Luo, B.; Gong, G.; Li, J.; Wang, J.; Wu, X.; Wen, J. Increases Urinary HMGA1 in Serous Epithelial Ovarian Cancer Patients. Cancer Biomark. 2015, 15, 325–331.

130. Stockley, J.; Akhand, R.; Kennedy, A.; Nyberg, C.; Crosbie, E.J.; Edmondson, R.J. Detection of MCM5 as a Novel Non-Invasive Aid for the Diagnosis of Endometrial and Ovarian Tumours. BMC Cancer 2020, 20, 1000.

131. Lee, S.-W.; Lee, H.-Y.; Bang, H.J.; Song, H.-J.; Kong, S.W.; Kim, Y.-M. An Improved Prediction Model for Ovarian Cancer Using Urinary Biomarkers and a Novel Validation Strategy. Int. J. Mol. Sci. 2019, 20, 4938.

132. Coticchia, C.M.; Curatolo, A.S.; Zurakowski, D.; Yang, J.; Daniels, K.E.; Matulonis, U.A.; Moses, M.A. Urinary MMP-2 and MMP-9 Predict the Presence of Ovarian Cancer in Women with Normal CA125 Levels. Gynecol. Oncol. 2011, 123, 295–300.

133. Ye, B.; Skates, S.; Mok, S.C.; Horick, N.K.; Rosenberg, H.F.; Vitonis, A.; Edwards, D.; Sluss, P.; Han, W.K.; Berkowitz, R.S.; et al. Proteomic-Based Discovery and Characterization of Glycosylated Eosinophil-Derived Neurotoxin and COOH-Terminal Osteopontin Fragments for Ovarian Cancer in Urine. Clin. Cancer Res. 2006, 12, 432–441.

134. Zhou, J.; Gong, G.; Tan, H.; Dai, F.; Zhu, X.; Chen, Y.; Wang, J.; Liu, Y.; Chen, P.; Wu, X.; et al. Urinary microRNA-30a-5p Is a Potential Biomarker for Ovarian Serous Adenocarcinoma. Oncol. Rep. 2015, 33, 2915–2923.

135. Sandow, J.J.; Rainczuk, A.; Infusini, G.; Makanji, M.; Bilandzic, M.; Wilson, A.L.; Fairweather, N.; Stanton, P.G.; Garama, D.; Gough, D.; et al. Discovery and Validation of Novel Protein Biomarkers in Ovarian Cancer Patient Urine. Prot. Clin. Appl. 2018, 12, 1700135.

136. Petri, A.L.; Simonsen, A.H.; Yip, T.-T.; Hogdall, E.; Fung, E.T.; Lundvall, L.; Hogdall, C. Three New Potential Ovarian Cancer Biomarkers Detected in Human Urine with Equalizer Bead Technology. Acta Obs. Gynecol. Scand. 2009, 88,

18–26.

137. Resnick, K.E.; Alder, H.; Hagan, J.P.; Richardson, D.L.; Croce, C.M.; Cohn, D.E. The Detection of Differentially Expressed microRNAs from the Serum of Ovarian Cancer Patients Using a Novel Real-Time PCR Platform. Gynecol. Oncol. 2009, 112, 55–59.
138. Ayaz, L.; Çayan, F.; Balci, Ş.; Görür, A.; Akbayir, S.; Yıldırım Yaroğlu, H.; Doğruer Unal, N.; Tamer, L. Circulating microRNA Expression Profiles in Ovarian Cancer. J. Obstet. Gynaecol. 2014, 34, 620–624.
139. Li, J.; Zhang, S.; Zou, Y.; Wu, L.; Pei, M.; Jiang, Y. miR-145 Promotes miR-133b Expression through C-myc and DNMT3A-mediated Methylation in Ovarian Cancer Cells. J. Cell Physiol. 2020, 235, 4291–4301.
140. Ren, X.; Zhang, H.; Cong, H.; Wang, X.; Ni, H.; Shen, X.; Ju, S. Diagnostic Model of Serum miR-193a-5p, HE4 and CA125 Improves the Diagnostic Efficacy of Epithelium Ovarian Cancer. Pathol. Oncol. Res. 2018, 24, 739–744.
141. Kan, C.W.; Hahn, M.A.; Gard, G.B.; Maidens, J.; Huh, J.Y.; Marsh, D.J.; Howell, V.M. Elevated Levels of Circulating microRNA-200 Family Members Correlate with Serous Epithelial Ovarian Cancer. BMC Cancer 2012, 12, 627.
142. Yokoi, A.; Matsuzaki, J.; Yamamoto, Y.; Yoneoka, Y.; Takahashi, K.; Shimizu, H.; Uehara, T.; Ishikawa, M.; Ikeda, S.; Sonoda, T.; et al. Integrated Extracellular microRNA Profiling for Ovarian Cancer Screening. Nat. Commun. 2018, 9, 4319.
143. Hamidi, F.; Gilani, N.; Belaghi, R.A.; Sarbakhsh, P.; Edgünlü, T.; Santaguida, P. Exploration of Potential miRNA Biomarkers and Prediction for Ovarian Cancer Using Artificial Intelligence. Front. Genet. 2021, 12, 724785.
144. Elias, K.M.; Fendler, W.; Stawiski, K.; Fiascone, S.J.; Vitonis, A.F.; Berkowitz, R.S.; Frendl, G.; Konstantinopoulos, P.; Crum, C.P.; Kedzierska, M.; et al. Diagnostic Potential for a Serum miRNA Neural Network for Detection of Ovarian Cancer. eLife 2017, 6, e28932.
145. Cirillo, P.D.R.; Margiotti, K.; Fabiani, M.; Barros-Filho, M.C.; Sparacino, D.; Cima, A.; Longo, S.A.; Cupellaro, M.; Mesoraca, A.; Giorlandino, C. Multi-Analytical Test Based on Serum miRNAs and Proteins Quantification for Ovarian Cancer Early Detection. PLoS ONE 2021, 16, e0255804.
146. Ali, F.T.; Soliman, R.M.; Hassan, N.S.; Ibrahim, A.M.; El-Gizawy, M.M.; Mandoh, A.A.Y.; Ibrahim, E.A. Sensitivity and Specificity of microRNA-204, CA125, and CA19.9 as Biomarkers for Diagnosis of Ovarian Cancer. PLoS ONE 2022, 17, e0272308.
147. Ge, L.; Sun, Y.; Shi, Y.; Liu, G.; Teng, F.; Geng, Z.; Chen, X.; Xu, H.; Xu, J.; Jia, X. Plasma circRNA Microarray Profiling Identifies Novel circRNA Biomarkers for the Diagnosis of Ovarian Cancer. J. Ovarian Res. 2022, 15, 58.
148. JMJ Piek; SGJG In ’t Veld; MG Best; B Tannous; A Supernat; CAR Lok; CD de Kroon; T Wurdinger EP457 Assessment of Ovarian Tumors with Tumor Educated Platelets (TEPs). Int. J. Gynecol. Cancer 2019, 29, A291.
149. Barrett, J.E.; Jones, A.; Evans, I.; Reisel, D.; Herzog, C.; Chindera, K.; Kristiansen, M.; Leavy, O.C.; Manchanda, R.; Bjørge, L.; et al. The DNA Methylome of Cervical Cells Can Predict the Presence of Ovarian Cancer. Nat. Commun. 2022, 13, 448.
150. Li, B.; Pu, K.; Ge, L.; Wu, X. Diagnostic Significance Assessment of the Circulating Cell-Free DNA in Ovarian Cancer: An Updated Meta-Analysis. Gene 2019, 714, 143993.
151. Singh, A.; Gupta, S.; Sachan, M. Evaluation of the Diagnostic Potential of Candidate Hypermethylated Genes in Epithelial Ovarian Cancer in North Indian Population. Front. Mol. Biosci. 2021, 8, 719056.
152. Singh, A.; Gupta, S.; Badarukhiya, J.A.; Sachan, M. Detection of Aberrant Methylation of HOXA9 and HIC1 through Multiplex METHYLIGHT Assay in Serum DNA for the Early Detection of Epithelial Ovarian Cancer. Int. J. Cancer 2020, 147, 1740–1752.
153. Liang, L.; Zhang, Y.; Li, C.; Liao, Y.; Wang, G.; Xu, J.; Li, Y.; Yuan, G.; Sun, Y.; Zhang, R.; et al. Plasma cfDNA Methylation Markers for the Detection and Prognosis of Ovarian Cancer. eBioMedicine 2022, 83, 104222.
154. Silwal-Pandit, L.; Langerød, A.; Børresen-Dale, A.-L. TP53 Mutations in Breast and Ovarian Cancer. Cold Spring Harb. Perspect. Med. 2017, 7, a026252.
155. Yang, W.-L.; Gentry-Maharaj, A.; Simmons, A.; Ryan, A.; Fourkala, E.O.; Lu, Z.; Baggerly, K.A.; Zhao, Y.; Lu, K.H.; Bowtell, D.; et al. Elevation of TP53 Autoantibody Before CA125 in Preclinical Invasive Epithelial Ovarian Cancer. Clin. Cancer Res. 2017, 23, 5912–5922.
156. Ma, Y.; Wang, X.; Qiu, C.; Qin, J.; Wang, K.; Sun, G.; Jiang, D.; Li, J.; Wang, L.; Shi, J.; et al. Using Protein Microarray to Identify and Evaluate Autoantibodies to Tumor-associated Antigens in Ovarian Cancer. Cancer Sci. 2021, 112, 537–549.
157. Hurley, L.C.; Levin, N.K.; Chatterjee, M.; Coles, J.; Muszkat, S.; Howarth, Z.; Dyson, G.; Tainsky, M.A. Evaluation of Paraneoplastic Antigens Reveals TRIM21 Autoantibodies as Biomarker for Early Detection of Ovarian Cancer in

Combination with Autoantibodies to NY-ESO-1 and TP53. Cancer Biomark. 2020, 27, 407–421.

158. Wang, P.; Qin, J.; Ye, H.; Li, L.; Wang, X.; Zhang, J. Using a Panel of Multiple Tumor-associated Antigens to Enhance the Autoantibody Detection in the Immunodiagnosis of Ovarian Cancer. J. Cell. Biochem. 2019, 120, 3091–3100.
159. Duan, Y.; Cui, C.; Qiu, C.; Sun, G.; Wang, X.; Wang, P.; Ye, H.; Dai, L.; Shi, J. Serum Autoantibodies against LRDD, STC1, and FOXA1 as Biomarkers in the Detection of Ovarian Cancer. Dis. Markers 2022, 2022, 6657820.
160. Pilyugin, M.; Ratajska, M.; Stukan, M.; Concin, N.; Zeillinger, R.; Irminger-Finger, I. BARD1 Autoantibody Blood Test for Early Detection of Ovarian Cancer. Genes 2021, 12, 969.
161. Qiu, C.; Duan, Y.; Wang, B.; Shi, J.; Wang, P.; Ye, H.; Dai, L.; Zhang, J.; Wang, X. Serum Anti-PDLIM1 Autoantibody as Diagnostic Marker in Ovarian Cancer. Front. Immunol. 2021, 12, 698312.
162. Mao, L.; Tang, Y.; Deng, M.; Huang, C.; Lan, D.; Nong, W.; Li, L.; Wang, Q. A Combined Biomarker Panel Shows Improved Sensitivity and Specificity for Detection of Ovarian Cancer. Clin. Lab. Anal. 2022, 36, e24232.
163. Fortner, R.T.; Damms-Machado, A.; Kaaks, R. Systematic Review: Tumor-Associated Antigen Autoantibodies and Ovarian Cancer Early Detection. Gynecol. Oncol. 2017, 147, 465–480.
164. Timmerman, D.; Valentin, L.; Bourne, T.H.; Collins, W.P.; Verrelst, H.; Vergote, I. Terms, Definitions and Measurements to Describe the Sonographic Features of Adnexal Tumors: A Consensus Opinion from the International Ovarian Tumor Analysis (IOTA) Group: Definitions for Sonography of Adnexal Tumors. Ultrasound Obs. Gynecol. 2000, 16, 500–505.
165. Ngu, S.F.; Chai, Y.K.; Choi, K.M.; Leung, T.W.; Li, J.; Kwok, G.S.T.; Chu, M.M.Y.; Tse, K.Y.; Cheung, V.Y.T.; Ngan, H.Y.S.; et al. Diagnostic Performance of Risk of Malignancy Algorithm (ROMA), Risk of Malignancy Index (RMI) and Expert Ultrasound Assessment in a Pelvic Mass Classified as Inconclusive by International Ovarian Tumour Analysis (IOTA) Simple Rules. Cancers 2022, 14, 810.
166. Jacobs, I.J.; Menon, U.; Ryan, A.; Gentry-Maharaj, A.; Burnell, M.; Kalsi, J.K.; Amso, N.N.; Apostolidou, S.; Benjamin, E.; Cruickshank, D.; et al. Ovarian Cancer Screening and Mortality in the UK Collaborative Trial of Ovarian Cancer Screening (UKCTOCS): A Randomised Controlled Trial. Lancet 2016, 387, 945–956.
167. Geomini, P.; Kruitwagen, R.; Bremer, G.L.; Cnossen, J.; Mol, B.W.J. The Accuracy of Risk Scores in Predicting Ovarian Malignancy: A Systematic Review. Obstet. Gynecol. 2009, 113, 384–394.
168. Hada, A.; Han, L.; Chen, Y.; Hu, Q.; Yuan, Y.; Liu, L. Comparison of the Predictive Performance of Risk of Malignancy Indexes 1–4, HE4 and Risk of Malignancy Algorithm in the Triage of Adnexal Masses. J. Ovarian Res. 2020, 13, 46.
169. Cui, R.; Wang, Y.; Li, Y.; Li, Y. Clinical Value of ROMA Index in Diagnosis of Ovarian Cancer: Meta-Analysis. Cancer Manag. Res. 2019, 11, 2545–2551.
170. Suri, A.; Perumal, V.; Ammalli, P.; Suryan, V.; Bansal, S.K. Diagnostic Measures Comparison for Ovarian Malignancy Risk in Epithelial Ovarian Cancer Patients: A Meta-Analysis. Sci. Rep. 2021, 11, 17308.
171. Oranratanaphan, S.; Wanishpongpan, S.; Termrungruanglert, W.; Triratanachat, S. Assessment of Diagnostic Values among CA-125, RMI, HE4, and ROMA for Cancer Prediction in Women with Nonfunctional Ovarian Cysts. Obstet. Gynecol. Int. 2018, 2018, 1–5.
172. Anderson, G.L.; McIntosh, M.; Wu, L.; Barnett, M.; Goodman, G.; Thorpe, J.D.; Bergan, L.; Thornquist, M.D.; Scholler, N.; Kim, N.; et al. Assessing Lead Time of Selected Ovarian Cancer Biomarkers: A Nested Case–Control Study. J. Natl. Cancer Inst. 2010, 102, 26–38.
173. Badgwell, D.; Bast, R.C., Jr. Early Detection of Ovarian Cancer. Dis. Markers 2007, 23, 309382.
174. Van Nagell, J.R.; Miller, R.W.; DeSimone, C.P.; Ueland, F.R.; Podzielinski, I.; Goodrich, S.T.; Elder, J.W.; Huang, B.; Kryscio, R.J.; Pavlik, E.J. Long-Term Survival of Women with Epithelial Ovarian Cancer Detected by Ultrasonographic Screening. Obstet. Gynecol. 2011, 118, 1212–1221.
175. Kobayashi, H.; Yamada, Y.; Sado, T.; Sakata, M.; Yoshida, S.; Kawaguchi, R.; Kanayama, S.; Shigetomi, H.; Haruta, S.; Tsuji, Y.; et al. A Randomized Study of Screening for Ovarian Cancer: A Multicenter Study in Japan. Int. J. Gynecol. Cancer 2008, 18, 414–420.
176. Pinsky, P.F.; Yu, K.; Kramer, B.S.; Black, A.; Buys, S.S.; Partridge, E.; Gohagan, J.; Berg, C.D.; Prorok, P.C. Extended Mortality Results for Ovarian Cancer Screening in the PLCO Trial with Median 15 Years Follow-Up. Gynecol. Oncol. 2016, 143, 270–275.
177. Lu, K.H.; Skates, S.; Hernandez, M.A.; Bedi, D.; Bevers, T.; Leeds, L.; Moore, R.; Granai, C.; Harris, S.; Newland, W.; et al. A 2-Stage Ovarian Cancer Screening Strategy Using the Risk of Ovarian Cancer Algorithm (ROCA) Identifies Early-Stage Incident Cancers and Demonstrates High Positive Predictive Value: Ovarian Cancer Screening Using ROCA. Cancer 2013, 119, 3454–3461.

178. Terry, K.L.; Schock, H.; Fortner, R.T.; Hüsing, A.; Fichorova, R.N.; Yamamoto, H.S.; Vitonis, A.F.; Johnson, T.; Overvad, K.; Tjønneland, A.; et al. A Prospective Evaluation of Early Detection Biomarkers for Ovarian Cancer in the European EPIC Cohort. Clin. Cancer Res. 2016, 22, 4664–4675.

179. Yu, M.; Stott, S.; Toner, M.; Maheswaran, S.; Haber, D.A. Circulating Tumor Cells: Approaches to Isolation and Characterization. J. Cell Biol. 2011, 192, 373–382.

180. Nguyen, D.X.; Bos, P.D.; Massagué, J. Metastasis: From Dissemination to Organ-Specific Colonization. Nat. Rev. Cancer 2009, 9, 274–284.

181. Agerbæk, M.Ø.; Bang-Christensen, S.R.; Yang, M.-H.; Clausen, T.M.; Pereira, M.A.; Sharma, S.; Ditlev, S.B.; Nielsen, M.A.; Choudhary, S.; Gustavsson, T.; et al. The VAR2CSA Malaria Protein Efficiently Retrieves Circulating Tumor Cells in an EpCAM-Independent Manner. Nat. Commun. 2018, 9, 3279.

182. Obermayr, E.; Castillo-Tong, D.C.; Pils, D.; Speiser, P.; Braicu, I.; Van Gorp, T.; Mahner, S.; Sehouli, J.; Vergote, I.; Zeillinger, R. Molecular Characterization of Circulating Tumor Cells in Patients with Ovarian Cancer Improves Their Prognostic Significance—A Study of the OVCAD Consortium. Gynecol. Oncol. 2013, 128, 15–21.

183. Yang, J.; Ma, J.; Jin, Y.; Cheng, S.; Huang, S.; Zhang, N.; Wang, Y. Development and Validation for Prognostic Nomogram of Epithelial Ovarian Cancer Recurrence Based on Circulating Tumor Cells and Epithelial–Mesenchymal Transition. Sci. Rep. 2021, 11, 6540.

184. Asante, D.-B.; Calapre, L.; Ziman, M.; Meniawy, T.M.; Gray, E.S. Liquid Biopsy in Ovarian Cancer Using Circulating Tumor DNA and Cells: Ready for Prime Time? Cancer Lett. 2020, 468, 59–71.

185. Rhim, A.D.; Mirek, E.T.; Aiello, N.M.; Maitra, A.; Bailey, J.M.; McAllister, F.; Reichert, M.; Beatty, G.L.; Rustgi, A.K.; Vonderheide, R.H.; et al. EMT and Dissemination Precede Pancreatic Tumor Formation. Cell 2012, 148, 349–361.

186. Marth, C.; Kisic, J.; Kaern, J.; Tropé, C.; Fodstad, Ø. Circulating Tumor Cells in the Peripheral Blood and Bone Marrow of Patients with Ovarian Carcinoma Do Not Predict Prognosis: Ovarian Carcinoma Cells in Bone Marrow and Peripheral Blood. Cancer 2002, 94, 707–712.

187. Judson, P.L.; Geller, M.A.; Bliss, R.L.; Boente, M.P.; Downs, L.S.; Argenta, P.A.; Carson, L.F. Preoperative Detection of Peripherally Circulating Cancer Cells and Its Prognostic Significance in Ovarian Cancer☆. Gynecol. Oncol. 2003, 91, 389–394.

188. Fan, T.; Zhao, Q.; Chen, J.J.; Chen, W.-T.; Pearl, M.L. Clinical Significance of Circulating Tumor Cells Detected by an Invasion Assay in Peripheral Blood of Patients with Ovarian Cancer. Gynecol. Oncol. 2009, 112, 185–191.

189. Aktas, B.; Kasimir-Bauer, S.; Heubner, M.; Kimmig, R.; Wimberger, P. Molecular Profiling and Prognostic Relevance of Circulating Tumor Cells in the Blood of Ovarian Cancer Patients at Primary Diagnosis and After Platinum-Based Chemotherapy. Int. J. Gynecol. Cancer 2011, 21, 822–830.

190. Suh, D.H.; Kim, M.; Choi, J.Y.; Bu, J.; Kang, Y.-T.; Kwon, B.S.; Lee, B.; Kim, K.; No, J.H.; Kim, Y.-B.; et al. Circulating Tumor Cells in the Differential Diagnosis of Adnexal Masses. Oncotarget 2017, 8, 77195–77206.

191. Rao, Q.; Zhang, Q.; Zheng, C.; Dai, W.; Zhang, B.; Ionescu-Zanetti, C.; Lin, Z.; Zhang, L. Detection of Circulating Tumour Cells in Patients with Epithelial Ovarian Cancer by a Microfluidic System. Int. J. Clin. Exp. Pathol. 2017, 10, 9599–9606.

192. Po, J.W.; Roohullah, A.; Lynch, D.; DeFazio, A.; Harrison, M.; Harnett, P.R.; Kennedy, C.; De Souza, P.; Becker, T.M. Improved Ovarian Cancer EMT-CTC Isolation by Immunomagnetic Targeting of Epithelial EpCAM and Mesenchymal N-Cadherin. J. Circ. Biomark. 2018, 7, 184945441878261.

193. Guo, Y.-X.; Neoh, K.H.; Chang, X.-H.; Sun, Y.; Cheng, H.-Y.; Ye, X.; Ma, R.-Q.; Han, R.P.S.; Cui, H. Diagnostic Value of HE4+ Circulating Tumor Cells in Patients with Suspicious Ovarian Cancer. Oncotarget 2018, 9, 7522–7533.

194. Kolostova, K.; Pinkas, M.; Jakabova, A.; Pospisilova, E.; Svobodova, P.; Spicka, J.; Cegan, M.; Matkowski, R.; Bobek, V. Molecular Characterization of Circulating Tumor Cells in Ovarian Cancer. Am. J. Cancer Res. 2016, 6, 973–980.

195. Abreu, M.; Cabezas-Sainz, P.; Alonso-Alconada, L.; Ferreirós, A.; Mondelo-Macía, P.; Lago-Lestón, R.M.; Abalo, A.; Díaz, E.; Palacios-Zambrano, S.; Rojo-Sebastian, A.; et al. Circulating Tumor Cells Characterization Revealed TIMP1 as a Potential Therapeutic Target in Ovarian Cancer. Cells 2020, 9, 1218.

196. Zhang, X.; Li, H.; Yu, X.; Li, S.; Lei, Z.; Li, C.; Zhang, Q.; Han, Q.; Li, Y.; Zhang, K.; et al. Analysis of Circulating Tumor Cells in Ovarian Cancer and Their Clinical Value as a Biomarker. Cell Physiol. Biochem. 2018, 48, 1983–1994.

197. Li, N.; Zuo, H.; Chen, L.; Liu, H.; Zhou, J.; Yao, Y.; Xu, B.; Gong, H.; Weng, Y.; Hu, Q.; et al. Circulating Tumor Cell Detection In Epithelial Ovarian Cancer Using Dual-Component Antibodies Targeting EpCAM And FRα. Cancer Manag. Res. 2020, 11, 10939–10948.

198. Wang, T.; Gao, Y.; Wang, X.; Tian, J.; Li, Y.; Yu, B.; Huang, C.; Li, H.; Liang, H.; Irwin, D.M.; et al. Establishment of an Optimized CTC Detection Model Consisting of EpCAM, MUC1 and WT1 in Epithelial Ovarian Cancer and Its Correlation with Clinical Characteristics. Chin. J. Cancer Res. 2022, 34, 95–108.
199. Haber, D.A.; Velculescu, V.E. Blood-Based Analyses of Cancer: Circulating Tumor Cells and Circulating Tumor DNA. Cancer Discov. 2014, 4, 650–661.
200. Jahr, S.; Hentze, H.; Englisch, S.; Hardt, D.; Fackelmayer, F.O.; Hesch, R.D.; Knippers, R. DNA Fragments in the Blood Plasma of Cancer Patients: Quantitations and Evidence for Their Origin from Apoptotic and Necrotic Cells. Cancer Res. 2001, 61, 1659–1665.
201. Underhill, H.R.; Kitzman, J.O.; Hellwig, S.; Welker, N.C.; Daza, R.; Baker, D.N.; Gligorich, K.M.; Rostomily, R.C.; Bronner, M.P.; Shendure, J. Fragment Length of Circulating Tumor DNA. PLoS Genet. 2016, 12, e1006162.
202. Huang, A.; Zhang, X.; Zhou, S.-L.; Cao, Y.; Huang, X.-W.; Fan, J.; Yang, X.-R.; Zhou, J. Plasma Circulating Cell-Free DNA Integrity as a Promising Biomarker for Diagnosis and Surveillance in Patients with Hepatocellular Carcinoma. J. Cancer 2016, 7, 1798–1803.
203. Thierry, A.R.; Mouliere, F.; Gongora, C.; Ollier, J.; Robert, B.; Ychou, M.; Del Rio, M.; Molina, F. Origin and Quantification of Circulating DNA in Mice with Human Colorectal Cancer Xenografts. Nucleic Acids Res. 2010, 38, 6159–6175.
204. García Moreira, V.; De La Cera Martínez, T.; Gago González, E.; Prieto García, B.; Alvarez Menéndez, F.V. Increase in and Clearance of Cell-Free Plasma DNA in Hemodialysis Quantified by Real-Time PCR. Clin. Chem. Lab. Med. (CCLM) 2006, 44, 1410–1415.
205. Zhu, J.W.; Charkhchi, P.; Akbari, M.R. Potential Clinical Utility of Liquid Biopsies in Ovarian Cancer. Mol. Cancer 2022, 21, 114.
206. Phallen, J.; Sausen, M.; Adleff, V.; Leal, A.; Hruban, C.; White, J.; Anagnostou, V.; Fiksel, J.; Cristiano, S.; Papp, E.; et al. Direct Detection of Early-Stage Cancers Using Circulating Tumor DNA. Sci. Transl. Med. 2017, 9, eaan2415.
207. Schmitt, M.W.; Fox, E.J.; Prindle, M.J.; Reid-Bayliss, K.S.; True, L.D.; Radich, J.P.; Loeb, L.A. Sequencing Small Genomic Targets with High Efficiency and Extreme Accuracy. Nat. Methods 2015, 12, 423–425.
208. Giannopoulou, L.; Chebouti, I.; Pavlakis, K.; Kasimir-Bauer, S.; Lianidou, E.S. RASSF1A Promoter Methylation in High-Grade Serous Ovarian Cancer: A Direct Comparison Study in Primary Tumors, Adjacent Morphologically Tumor Cell-Free Tissues and Paired Circulating Tumor DNA. Oncotarget 2017, 8, 21429–21443.
209. Wang, B.; Yu, L.; Luo, X.; Huang, L.; Li, Q.-S.; Shao, X.-S.; Liu, Y.; Fan, Y.; Yang, G.-Z. Detection of OPCML Methylation, a Possible Epigenetic Marker, from Free Serum Circulating DNA to Improve the Diagnosis of Early-Stage Ovarian Epithelial Cancer. Oncol. Lett. 2017, 14, 217–223.
210. Wu, Y.; Zhang, X.; Lin, L.; Ma, X.-P.; Ma, Y.-C.; Liu, P.-S. Aberrant Methylation of RASSF2A in Tumors and Plasma of Patients with Epithelial Ovarian Cancer. Asian Pac. J. Cancer Prev. 2014, 15, 1171–1176.
211. Zhang, Q.; Hu, G.; Yang, Q.; Dong, R.; Xie, X.; Ma, D.; Shen, K.; Kong, B. A Multiplex Methylation-Specific PCR Assay for the Detection of Early-Stage Ovarian Cancer Using Cell-Free Serum DNA. Gynecol. Oncol. 2013, 130, 132–139.
212. Dong, R.; Yu, J.; Pu, H.; Zhang, Z.; Xu, X. Frequent SLIT2 Promoter Methylation in the Serum of Patients with Ovarian Cancer. J. Int. Med. Res. 2012, 40, 681–686.

Retrieved from https://encyclopedia.pub/entry/history/show/124038