Screening Prospects for Ovarian Cancer

Subjects: Oncology

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Ovarian cancer (OC) has the highest mortality rate of all gynecologic malignancies. The overall five-year survival is 46% and varies depending on the stage and histological type of the tumor. High-grade serous carcinoma (HGSOC) accounts for 75% of all epithelial ovarian malignancies and is diagnosed mainly at FIGO stage III (51%) or IV (29%), reflecting the aggressive nature.

ovarian cancer liquid biopsies uterine lavage high-throughput methods NGS-based multigene panels

1. Introduction

Ovarian cancer (OC) has the highest mortality rate of all gynecologic malignancies [1]. The overall five-year survival is 46% and varies depending on the stage and histological type of the tumor [2]. High-grade serous carcinoma (HGSOC) accounts for 75% of all epithelial ovarian malignancies and is diagnosed mainly at FIGO stage III (51%) or IV (29%), reflecting the aggressive nature [3]. In contrast, nonepithelial and more rare epithelial tumors such as endometrioid, mucinous. and clear-cell carcinomas are more frequently diagnosed at FIGO stages I–II [3]. Consequently, the five-year survival for HGSOC is 43%, compared with 82%, 71%, and 66% for endometrioid, mucinous, and clear-cell carcinoma, respectively. The five-year OS rate is only 9% for FIGO stage IV HGSOC patients [1].

Until recently, OC classification was based on morphology and immunohistochemistry (IHC), but more modern diagnostic approaches take into account molecular genetics, protein post-translational transformations, and immune cell infiltrates [4][5]. Over the last few decades, two distinct pathogenesis models were defined dividing ovarian malignancies into ovarian-origin OC and extra ovarian-origin OC. Ovarian-origin malignancies are very rare, mostly occurring at a young age or in childhood, and are presented by two main groups: (1) sex-cord stromal tumors tend to manifest as low-grade disease with a nonaggressive clinical course and are usually diagnosed at the early stages; (2) predominantly malignant germ cell tumors stand out due to their very fast tumor growth and the progression of clinical symptoms. Therefore, detailed screening tests do not seem mandatory for this category of tumors. The majority of epithelial ovarian cancers (EOCs) and epithelial–stromal ovarian tumors are suspected to be of extra ovarian origin, as the derivative cell is not ovarian (serous, mucinous, endometrioid, clear cell, and others). For clinical decision making, surface epithelial malignancies were further divided into two categories as a function of their pathogenetic pathways: type I and type II [2][6][7][8].

Most malignant tumors of the ovary are surface epithelial (90%). In 2014, the World Health Organization (WHO) recognized five principal epithelial OC histotypes: high-grade serous, low-grade serous, endometrioid, clear cell, and mucinous carcinoma. Other malignancies such as carcinosarcoma, adenosarcoma, and endometrioid stromal sarcoma are very rare; therefore, there is very little data concerning their pathogenesis and molecular features. Moreover, not otherwise specified ovarian tumors such as neuroendocrine, rete ovarii adenocarcinoma, Wilm's tumor, and others are exceptionally rare with an incidence of less than 0.1%. The most frequent mutation characteristics according to tumor morphology are presented in **Table 1** [6][7][8][9][10][11][12][13][14][15][16][17][18][19].

Table 1. Discriminatig features of major histotypes of epithelial ovarian cancer.

Histology	Cells of Origin	Precursors	More Frequent Somatic Mutations
Low-Grade Serous Carcinoma	Fallopian tube progenitor cell or secretory cell	Serous cystadenoma, adenofibroma, atypical proliferative serous tumor, noninvasive micropapillary serous borderline tumor	KRAS (30%), BRAF (30%), NRAS, EIF1AX, USP9X, ERBB2, FRAR1, NF1, HRAS
Mucinous Carcinoma	Unknown	Mucinous adenoma, mucinous borderline tumor	CDKN2A (76%), KRAS and TP53 (both 64%), ERBB2 (26%), RNF43, BRAF, PIK3CA, ARID1A (8–12%)
Endometrioid Carcinoma	Endometrial epithelial cells	Endometriosis and endometrial cell-like hyperplasia, endometrioid borderline tumor	ARID1A (30%), PIK3CA (30%), TERT, CTNNB1, TP53
Clear-Cell Carcinoma	Endometrial epithelial cells	Endometriosis, endometrioid borderline tumors	PIK3CA (50%), ARID1A (50%), KRAS, MET, PTEN, CTNNB1, RPL22, TP53
High-Grade Serous Carcinoma	Fallopian tube progenitor cell or secretory cell	SCOUT, P53 signature, STIC	TP53 (96–98%) BRCA1/BRCA2 (10%, 25% somatic + germline); CNAs of CCNE1 amplification, PTEN deletion, RB1 and NF1 loss
Carcinosarcomas	Unknown	Carcinomatous component	TP53, CTNNB1

STIC—serous tubal intraepithelial carcinomas, SCOUT—secretory cell OUT growth.

Type II tumors are characterized as highly aggressive neoplasms accounting for 75% of all EOCs, which are usually diagnosed at a late stage. They include high-grade serous carcinoma (HGSOC)—the most common type—and rare types such as high-grade endometrioid, undifferentiated carcinomas, and malignant epithelial mesenchymal tumors (carcinosarcomas). Type II ovarian tumors have a high level of genetic instability; the majority harbors TP53 mutations [6][7][8]. Recent data suggest that HGSOC tumors originate from the epithelium of

the fallopian tube. Mutation of TP53 is the first known molecular event in the transformation of fallopian tube secretory cells to serous tubal intraepithelial carcinomas (STICs), which leads to HGSOC initiation. Mutated TP53 can be identified as an early tumor precursor of HGSOC. It has been estimated that it takes approximately seven years from STIC to clinically evolve into HGSOC [6][17][20]. Almost 80% of women present with advanced (stages III-IV) disease and poor prognosis (the five-year survival rate is around 25%). Since up to 98% of all HGSOC cases are characterized by TP53 somatic mutations, this biomarker is widely investigated as a potential diagnostic tool for OC diagnostics [6][8][17][19].

2. Materials and Methods

We performed a literature search in NCBI PubMed from January 2014 to September 2020 with a specific emphasis on liquid biopsy biomarkers for early OC detection. We used the keywords “ovarian cancer” together with “circulating free DNA”, ”circulating tumor DNA”, ”circulating tumor cells”, “small non coding RNA”, “microRNA”, “PIWI- interactingRNA”, “Transfer-RNA-derivated small RNA”, “liquid biopsy”, “TEPS”, and “uterine lavage”. We identified 2193 abstracts in NCBI PubMed and selected 30 reports considered inclusion criteria—evaluating the efficacy of liquid biopsies as a diagnostic tool for OC detection. We summarize the results of these studies in **Table 2** . This work provides deeper understanding of the aspects of OC pathogenesis and existing challenges for liquid biopsy applications in clinical practice.

able 2. Studies on ctDNA, DNA, CTC and microRNA in ovarian cancer.

Author (Year), References	Number of OC Patients	Specimen	Method	Genetic Marker/Antigen	Detection Rate (%)	Detection Rate (%) (I-II Stage)	Sensitivity (%)	Specificity (%)
K.K Lin et al. (2019) [21]	112 germline or somatic BRCA-mutant HGOC	Plasma (ctDNA)	Targeted-NGS	BRCA1, BRCA2, TP53	96 for TP53	NR	NR	NR
Y. Wang et al. (2018) [22]	83 OC	Plasma (ctDNA)	Pap SEEK-PCR-based error-reduction technology Safe-SeqS	18 genes + assay for aneuploidy	43	35	NR	100
Y. Wang et al. (2018) [22]	83 OC	Plasma (ctDNA) + Pap Brush samples	Pap SEEK-PCR-based error-reduction technology Safe-SeqS	18 genes + assay for aneuploidy	63	54	NR	100

Author (Year), References	Number of OC Patients	Specimen	Method	Genetic Marker/Antigen	Detection Rate (%)	Detection Rate (%) (I-II Stage)	Sensitivity (%)	Specificity (%)
P.A. Cohen et al. (2018) [23]	54 OC	Plasma (ctDNA) + proteins	CancerSEEK Targeted NGS	16 gene panel + 41 protein biomarkers	98	38	NR	>99 AUC = 0.91
J. Phallen et al. (2017) [24]	42 OC	Plasma (ctDNA)	Targeted NGS (TEC-Seq) and ddPCR	55 gene panel	71	68	NR	100
E. Pereira et al. (2015) [25]	22 HGSOC	Serum (ctDNA)	ddPCR, NGS, WES	TP53, PTEN, PIK3CA, MET, KRAS, FBXW7, BRAF	93.8	NR	81-91	60-99
A. Piskorz et al. (2016) [25]	18 OC	Plasma (ctDNA)	Targeted NGS	TP53	100	NR	NR	NR
R.C. Arend et al. (2018) [26]	14 OC	Plasma (cfDNA)	Targeted NGS	50 gene	100	NR	NR	NR
J.D. Cohen et al. (2016) [27]	32 HGSOC	Plasma cfDNA (instability)	WEG (WISECONDOR)	CNV	38	40.6	NR	93.8
A. Vanderst-ichele et al. [28]	57 OC and bordline tumors	Plasma cfDNA	WGS	CNV	67	NR	NR	99.6 AUC = 0.89
Y. Wang et al. (2018) [22]	245 OC	Cervix Pap brush samples (DNA)	Pap SEEK-PCR-based error-reduction technology Safe-SeqS,	18 genes + assay for aneuploidy	NR	33	34	99
		Tao Brush (DNA)	Pap SEEK-PCR-based error-reduction technology Safe-SeqS	18 genes + assay for aneuploidy	NR	45	47	100
Salk et al. (2019) [29]	10 OC	Uterine lavage (DNA)	Duplex Sequencing	TP53	80	NR	70	100
E.Maritschnegg (2018) [30]	33 OC	Uterine lavage (DNA)	Deep-sequencing	AKT1, APC, BRAF, CDKN2A, CTNNB1,	80 for TP53	NR	NR	NR

Author (Year), References	Number of OC Patients	Specimen	Method	Genetic Marker/Antigen	Detection Rate (%)	Detection Rate (%) (I-II Stage)	Sensitivity (%)	Specificity (%)
				EGFR, FBXW7, FGFR2, KRAS, NRAS, PIK3CA, PIK3R1, POLE, PPP2R1A, PTEN, TP53				
E.Maritschnegg (2015) [31]	30 OC	Uterine lavage (DNA)	Massively parallel sequencing	AKT1, APC, BRAF, CDKN2A, CTNNB1, EGFR, FBXW7, FGFR2,	60 for TP53	100 for TP53	NR	NR
			With ddPCR and SafeSeqS	KRAS, NRAS, PIK3CA, PIK3R1, POLE, PPP2R1A, PTEN, TP53	80 for TP53			
B.K Erickson et al. (2014) [32]	5 OC	Vaginal tampon (DNA)	Massively parallel sequencing	NR	60	NR	60	NR
Kinde et al. (2013) [33]	22 OC	Liquid Pap smear tests (DNA)	Massively parallel sequencing	NR	41	NR	NR	NR
N. Li et al (2019) [34]	30 EOC	Plasma (CTC)	Magnetic nanospheres (MNs) + IHC	EpCAM, FRα	92	NR	75	90 AUC = 0.8
Zhang et al. (2018) [35]	109 EOC	Plasma (CTC)	Imunomagnetic beads (EpCAM, HER2 and MUC1) + multiplex RT-PCR	EpCAM, HER2, MUC1, WT1, P16, PAX8	90	93	NR	NR
Q Rao et al. (2017) [36]	23 EOC	Plasma (CTC)	Microfluidic system with immunomagnetic	EpCAM, CK3-6H5, panCK	87	NR	NR	NR

Author (Year), References	Number of OC Patients	Specimen	Method	Genetic Marker/Antigen	Detection Rate (%)	Detection Rate (%) (I-II Stage)	Sensitivity (%)	Specificity (%)
			beads (EpCAM) + IHC					
M. Lee et al. (2017) [37]	54 EOC	Plasma (CTC)	Incorporating a nanoroughened microfluidic platform + IHC	EpCAM, TROP-2, EGFR, Vimentin, N-cadherin	98.1	NR	NR	NR
Dong Hoon Suh et al. (2017) [38]	87 EOC, bordline, benigh	Plasma (CTC)	Tapered-slit membrane filters + IHC	EpCAM, CK9	56.3	NR	77.4	55.8 AUC = 0.61–0.75
I. Chebouti et al. (2017) [39]	95 EOC	Plasma (CTC)	Adna Test Ovarian Cancer and EMT-1 Select/Detect + Multiplex RT-PCR	EpCAM, ERCC1, MUC1, MUC16, PI3Ka, Akt-2, Twist	82	NR	>90	>90
K. Kolostova et al. (2016) [40]	40 OC	Plasma (CTC)	MetaCell + IHC/qPCR	ICC: NucBlueTM, CelltrackerTM. EpCAM, MUC1, MUC16, KRT18, KRT19, ERCC1, WT1	58	NR	NR	NR
K. Kolostova et al (2015) [41]	118 OC	Plasma (CTC)	MetaCell + IHC/qPCR	ICC: NucBlueTM, CelltrackerTM. EpCAM, MUC1, MUC16, KRT18, KRT19,	65.2	NR	NR	NR
M. Pearl et al. (2015) [42]	31 EOC	Plasma (CTC)	CAM uptake-cell enrichment + IHC/RT-qPCR	EpCAM, Ca 125, CD44, seprase EpCAM, CD44, MUC16, FAP	100	NR	83	97
Pearl et al. (2014) [43]	129 EOC	Plasma (CTCs)	CAM uptake – cell enrichment +	EpCAM, Ca 125, CD44,	88. 6	41.2	83	95.1

Author (Year), References	Number of OC Patients	Specimen	Method	Genetic Marker/Antigen	Detection Rate (%)	Detection Rate (%) (I-II Stage)	Sensitivity (%)	Specificity (%)
			IHC	seprase				
Gao et al. (2015) [44]	143 all 74 EOC	Serum microRNA	qRT-PCR	miR-200c	NR	NR	72	70, AUC = 0.79
				miR-141			69	72, AUC = 0.75
Meng et al. (2016) [45]	163 EOC	Serum microRNA	TaqMan microRNA assays and ELISA	miR-200a	NR	NR	83	90, AUC = 0.91
				miR-200b			52	100, AUC = 0.81
				miR-200C			31	100, AUC = 0.65
				3miRNAs set			88	90, AUC = 0.92
Yokoi et al. in (2017) [46]	269 all 155EOC	Serum microRNA	qRT-PCR + statistical cross-validation methods	8 miRNA combination	NR	86	92	91, AUC = 0.96
Yokoi et al. in (2018) et al. [47]	EOC 333	Serum microRNA	Microarrays	10 miRNAs set miRNA-320a, -665, -1275, -3184-5p, -3185, -3195, -4459, 4640-5p, -6076, and -6717-5p. EOS vs. non cancer	NR	NR	99	100, AUC = 0.72–1.0
Kim S. (2019) [48]	68 all 39HGOC	Serum microRNA	qRT-PCR	miRNA-145	NR	NR	91.7	86.8, AUC = 86.8
				miRNA-200C			72.9	90.0, AUC = 77.9

NR: not reported; OC- ovarian cancer; EOC: epithelial ovarian cancer; ddPCR: Droplet digital PCR; RT-PCR: real time PCR technology; qRT-PCR: quantitative real time PCR; NGS: next generation sequencing; CAM: cell adhesion matrix; WES: whole exome sequencing; TGS: targeted gene sequences; HGSOC: high grade serous

ovarian cancer; ddPCR: droplet digital PCR; AUC- areas under the ROC curves; IHC: immunocytochemistry staging; CNV: Copy number variation; WES: Whole exome sequencing; Safe-SeqS: Safe-sequencing system; WGS: Whole genome sequencing.

3. Modern Means for Early Detection of OC

An approach for the lavage of the uterine cavity to detect cancer cells that have been shed was developed by Paul Speiser, Professor at Medical College of Vienna, and colleagues [49].

A study published by Kinde et al. in 2013 analyzed the liquid Pap test from the uterine cervix for detecting ovarian and uterine cancers. Massively parallel sequencing for tumor-specific mutations using a 12-gene panel was performed on DNA extracted from liquid Pap smear tests. This technique was successfully applied to 100% of patients. Detectible DNA mutations were found in 24 (100%) for endometrial cancer patients and in 9 of 22 (41%) OC, mainly in late stages [50]. A pilot study showed that tumor cells and fragments containing tumor DNA can be found and collected in the vagina using a vaginal tampon and studied by using genetic analysis. They succeeded in revealing TP53 mutations in 60% of advanced HGSOCs [51]. Y. Wang et al. 2018 published data of DNA analysis in Pap brush samples from 245 OC patients, and the detection sensitivity was 33%, including 34% for patients with stage I–II disease [52].

PIWI-interacting RNA (piRNAs) interact with PIWIs—germline-specific Ago family nuclear RNA-binding proteins—and form piRNA-induced silencing complexes (piRISCs). The latest data demonstrate the contribution of piRNAs and PIWI proteins to the main carcinogenesis events: cell proliferation, resisting cell death, genome instability, invasion, and metastasis. PIWIs are essential for germline tissues and gametogenesis. Due to their restricted expression in reproductive tissue and tumors, PIWIs are classified as cancer/testis antigens (CTA). They are considered as excellent objects for diagnostic/prognostic biomarkers and targeted therapies. piRNAs regulate mechanistic RNA-based inhibition of transposable elements in germlines. They can target nontransposable elements as well—such as protein-coding messenger RNAs (mRNAs) —and modulate their expression, not only in germlines, but also in somatic cells, by a mechanism similar to that of miRNAs. piRISCs contribute to cancer development and progression by promoting a stem-like state of cancer cells, or cancer stem cells. The expression of germline genes in cancer reflects the ectopic activation in somatic tissues of a naturally silenced developmental program managing the escape from cell death, immune circumvention, and invasiveness [53][54]. In gynecologic malignancies, the study of piRNA pathophysiological significance, expression levels, and diagnostic performance remains exploratory.

Extracellular vesicles (EVs) contain cell surface proteins, as well as miRNAs and other molecules. EV-associated proteins and lncRNAs were investigated as potential biomarkers and showed greater sensitivity comparing to conventional biomarkers, but there are no data about the value to OC patients [55][56][57][58].

4. Conclusions and Future Prospects

Innovative technologies based on very small samples are likely to drastically change medical practice in the near future. Presently available liquid biopsy assessments are not ready for use in clinical practice. Significant efforts remain to create reliable tests for early OC detection. Uterine lavage techniques are easy to apply and safe, and this approach appears very promising for implementation in daily clinical practice. miRNAs are promising biomarkers for cancer diagnosis and prognosis, and large-scale prospective clinical studies are ongoing. Research efforts directed toward single-cell analysis are likely to shed more light on diagnostic biomarkers and potential therapeutic targets in the future.

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