

Monitoring Biomarkers for Ovarian Cancer Recurrence

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Abstract Late diagnosis of ovarian cancer is associated with a high mortality rate among all gynecologic tumors in most industrialized countries of the world. Even if initial treatment of ovarian cancer is successful, most patients experience tumor recurrence within the next two years, eventually leading to death. Due to the high probability of recurrence and poor prognosis after recurrence, there is an urgent need to predict disease progression as early as possible in order to find strategies to detect and prevent recurrence. Our aim in this review is to discuss recent data published in the literature on the use of biomarkers to detect ovarian cancer recurrence. Biomarkers aid in the management of ovarian cancer by helping to distinguish between benign and malignant masses in the pelvis and monitoring response to treatment. To assess their potential as prognostic biomarkers of ovarian cancer recurrence, we analyzed all data on the most commonly used biomarkers, including CA 125, HE4, and their combinations, as well as tumor-associated DNA. It is still unclear how to accurately predict and estimate the likelihood of disease recurrence. **Purpose of the study:** to review information on the potential use of biomarkers for monitoring and diagnosing ovarian cancer recurrence. Sources for research literature from the past ten years can be found in the databases of PubMed and E-library. Combinations of the text terms "ovarian neoplasm," "ovarian cancer," "ovarian malignancy," "recurrence," and "ovarian biomarkers" were used to find studies. Reviews of publications on the role of ovarian biomarkers in females experiencing ovarian cancer recurrence were among the selection criteria for this descriptive review. The biomarkers of ovarian cancer that have been studied the most are HE-4 and CA 125. Along with well-established clinical and morphologic prognostic factors, these biomarkers play a significant role in the development of ovarian cancer recurrence and offer insight into the disease's course and prognosis prediction. A non-invasive liquid biopsy can identify circulating tumor DNA (ctDNA), a promising biomarker of ovarian cancer. ctDNA has been shown to have a wide range of applications in tracking ovarian carcinoma during patient diagnostic and prognostic evaluations. It is also being integrated into clinical trials for disease evaluation. Furthermore, to help in early detection, ctDNA analysis can be combined with a number of "omics" techniques to examine proteins, RNA, nucleosomes, exosomes, related immune markers, and epigenetics. Nonetheless, a number of biological and technical challenges have made ctDNA analysis less useful. The detection of ctDNA is hampered by certain intrinsic features, such as methylation, length, and copy number variation, which could improve the biomarker's usefulness. Before ctDNA assays can be developed for clinical use, these problems need to be resolved because they have a lot of potential as a test for cancer screening. In addition to discussing our opinions of clinical trials targeted at treating this difficult type of cancer, this review concentrates on studies regarding the possible clinical application of ctDNA in the diagnosis of ovarian cancer. While the literature has identified several biomarkers of response to different agents in ovarian cancer, most of them lack high-level evidence. This report emphasizes the unfulfilled need for prognostic biomarker identification and validation to direct treatment and future study design in ovarian cancer.

Keywords Ovarian cancer (OC), Cancer antigen (CA 125), Human epididymis protein 4 (HE-4), Digital droplet polymerase chain reaction (ddPCR), Circulating tumor DNA (ctDNA)

1. Introduction

Ovarian cancer (OC) is one of the 10 cancers with the highest incidence and mortality rates among women standardized worldwide. [1]. Ovarian cancer has a lower incidence rate than breast cancer, but has a three times higher mortality rate and a worse prognosis [2]. Due to the

projected increase in the global cancer burden, death from ovarian cancer are expected mortality is expected to increase by 47% worldwide by 2040 [3,4]. The lack of early screening programs and specific diagnostic markers makes early diagnosis difficult [5]. In the early stages of the disease, non-specific symptoms or none at all, which contributes to the high mortality rate of patients with ovarian cancer [4]. According to the International Federation of Gynecologists and Obstetricians' classification, 90% of ovarian cancers are detected at stages I and II, with a 5-year survival rate of 70%. However, in advanced stages (III and IV), FIGO reports survival rate approaching 30%. Recurrence is common

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Received: Apr. 8, 2024; Accepted: May 5, 2024; Published: May 7, 2024

Published online at <http://journal.sapub.org/ajmms>

in approximately 60% of patients with ovarian cancer in remission. Therefore, it is important to continuously evaluate new treatment modalities such as potential potential chemotherapeutic agents, targeted immunotherapy and combinatorial approaches such as poly-DNA-ribose polymerase inhibitors and anti-angiogenic factors [6,7].

In the last two decades, cancer research has progressed and gained international attention. Histopathologic aspects of ovarian cancer and related modern molecular therapies are constantly evolving and require a constant review of the literature. Tumor classification (G1-G3) and the histological grade of ovarian cancer are two of the many factors that determine which treatment is best [8]. Platinum-based chemotherapy and surgery offer a good prognosis for almost 80% of patients, but for the remaining patients other treatment approaches should be considered [9,10]. Although staging after surgical resection is the most effective treatment option, patients with advanced disease who generally have a poor long-term survival prognosis often need neoadjuvant therapy. Studies have shown that patients who receive chemotherapy and undergo complete resection of the visible tumor have the best and most stable outcome [10].

The vast majority of ovarian tumors are of epithelial in origin and are divided into two subtypes of prognostic value, types I and II. Approximately 30% of ovarian cancers are classified as type I, which includes less aggressive serous, endometrioid, clear cell, and mucinous carcinomas. Type I cancers are usually genetically stable and slow growing. 70% of ovarian cancers are type II, which includes more aggressive, genetically unstable and highly malignant serous carcinomas. This classification may help explain why tumor cells can escape immune clearance and spread cancer [11].

Due to the high probability of recurrence and unfavorable prognosis after recurrence, it is important to predict early progression and develop strategies for prevention and early detection of recurrence [12]. Biomarkers such as human epididymis protein 4 (HE4) and cancer antigen 125 (CA-125) help identify ovarian cancer. Common detection methods for ovarian cancer detection include transvaginal ultrasonography and CA-125 detection. The risk of Malignancy Index (RMI) and the Risk of Malignancy Algorithm (ROMA) to distinguish between benign from malignant disease [13]. Many biomarkers have been developed over the years and more and more studies are combining CA125 with different biomarkers. However, none of the biomarkers, such as carcinoembryonic antigen (CEA), are used in clinical practice for the early detection of OC. CA125, carbohydrate antigen 19-9 (CA19-9) and HE4 are effective due to their low sensitivity and specificity [14].

Since biomarkers are found in patient samples, their sensitivity is determined by how well they identify diseased patients, while their specificity is determined by how well they remain undetected in healthy individuals [15]. Only one of these characteristics in a biomarker can lead to false positives or false negatives, respectively [15]. The main objective of the data obtained was to determine the association between serum biomarkers and the risk of ovarian cancer recurrence.

As a result, biomarkers can be used as a prognostic indicators to differentiate patients according to their risk of following a particular outcome and to predict the likelihood of disease recurrence [14]. To date, one of the most widely used tumor biomarkers in routine clinical practice for disease surveillance is the measurement of serum CA125 levels, which is used to detect clinical signs of recurrence [16]. Sensitive biomarkers that can predict ovarian cancer recurrence over a long enough period of time for CA125 to be elevated are urgently needed so that patients can benefit from early drug therapy that can prolong recurrence-free survival and improve overall survival. The aim of this descriptive review is to explore the role of biomarkers in the early detection of ovarian cancer recurrence and summarize the available evidence. Serum biomarkers are increasingly being investigated and non-invasive methods for early detection of ovarian cancer recurrence are needed. Several biomarkers may be important for the development of effective ovarian cancer management in the early detection of recurrence, which is associated with a higher response rate to drug therapy [18]. The development of effective strategies to control ovarian cancer at the recurrence level appears to rely on various biomarkers for the early detection of recurrence at the stage when response rates to drug therapy are high [18]. The most frequently cited biomarkers are summarized here.

2. Diagnostic Biomarkers Cancer Antigen (CA125)

CA125, a nonspecific marker, belongs to the mucin family of glycoproteins that are frequently expressed in müllerian and derived coelomic epithelial tissue [19]. The most typical application of this biomarker is in ovarian lesions. When Bast *et al.* [20] identified the monoclonal antibody OC125 in cancerous ovarian tissue as opposed to healthy ovarian tissue, it was applied in the early 1980s. In patients who are pre- or postmenopausal, its upper limit is 35 U/mL [21]. Taking into account that some benign conditions, including endometriosis, pelvic inflammatory disease, and peritonitis, may cause its levels to rise. But also in malignant conditions such as ovarian cancer [22]. One of the most popular biomarkers for prognostic prediction and epithelial ovarian cancer (EOC) surveillance in routine clinical practice is CA125. Though it may also be latent, as in about 20% of ovarian cancers [24], a serum CA125 concentration >35 U/mL typically indicates potential malignancy: an elevation of 47% in early-stage EOC and an elevation of 80–90% in late-stage EOC [23]. Levels over 65 U/mL of CA125, which is used for cancer surveillance, are linked to a lower 5-year survival rate [25]. When utilizing the tumor marker CA125 alone, with a threshold of 35 U/mL, the sensitivity and specificity of identifying an early ovarian cancer recurrence were 67.39% and 86.79%, respectively [26]. A halving of CA125 values after treatment is typically associated with a favorable response to treatment, whereas a doubling of values indicates drug resistance or disease progression. CA125 appears to be

suggestive when comparing its pre-treatment levels with post-treatment levels. An elevation above the threshold of 35 U/mL can be considered a suspicion of progression or relapse in patients whose CA125 values never normalize or in patients whose CA125 values normalize after treatment [27].

A retrospective analysis of 342 patients with surgically treated ovarian cancer reveals that the median CA125 value among patients who developed recurrence is 35 U/mL (29.7 U/mL). PET examination revealed lesions in the spleen, liver, and pelvic region in three patients with CA125 values of 14.5, 13.5, and 20.9 U/mL, respectively [17]. As a result, a 10.5% increase in CA 125 levels may be indicative of disease progression and necessitates a CT scan. Changes of less than 0.5% indicate a lack of progression. If the changes are between 0.5% and 10.5%, an individualized clinical approach is recommended [28]. CA125 elevation appears 3-5 months before the appearance of recurrence signs and symptoms in 70% of cases [29]. These data are in line with earlier studies [30–32], which have shown that although CA125 has a high specificity for detecting recurrences, its sensitivity is low because not all ovarian cancers, especially ovarian mucinous cancer [33], have elevated blood CA125 levels. Several authors, however, disagree with these results and contend that patients who have undergone epithelial OC surgery should not have their CA125 levels monitored as it is not clinically relevant for that purpose [34]. For instance, Rustin et al. showed that early treatment of ovarian cancer recurrence based on CA125 elevation did not improve overall survival when compared to treatment for clinical recurrence. However, this study has several limitations: only chemotherapy was considered as early treatment, and the impact of second-line cytoreductive surgery was not considered; CA125 changes within the normal range were not considered, delaying the detection of relapse; and in some cases, suboptimal therapy by current standards was used [35]. The European Society of Gynecologic Oncology (ESGO) and the European Society of Medical Oncology (ESMO) both recommended, in part, that regular CA125 measurements not be universally discarded in the routine follow-up of all patients with ovarian cancer based on this single randomized trial. This is because regular CA125 measurements may signal tumor growth in some patients before symptoms appear [36,37]. Out of all the biomarker panels intended to offer optimal surveillance for ovarian cancer, CA125 has drawn the greatest attention thus far. Increased CA125 levels during surveillance have been linked to specific recurrence localization at the peritoneal and intra-abdominal levels (lymph node, vaginal stump, and cul-de-sac) [38]. Individuals with brain and supradiaphragmatic lung lesions did not appear to have elevated CA125 levels (35 U/mL).

3. Human Epididymis Protein 4 (HE4)

Protease inhibitor HE4 [39] is mainly expressed in the respiratory and reproductive tracts, but it is also highly

sensitive and specific in identifying overexpression of certain cancerous cells (OC), especially endometrioid (100% overexpression) and serous subtypes (93% overexpression) [40]. By triggering the EGFR/MAPK signaling pathway, HE4 has been shown to control tumor cell adhesion, migration, and growth [41]. The FDA has authorized HE4 for the purpose of tracking the advancement or recurrence of EOC syndrome [42]. What seems interesting is that HE4 seems to be able to predict OC recurrence to CA125 in some patients and may be elevated in patients whose tumors do not express CA125 [43], and Laskshmann et al. found that serum HE4 had equivalent sensitivity (85.3% vs. 84.3%) but higher specificity (91.4% vs. 70.2%) than serum CA125 in detecting recurrence and a wait time of 3 months compared to CA125 [44]. Liao et al. [46] found that urine HE4 levels became positive prior to clinical relapse in several women despite normal serum HE4 and CA-125 levels. Anastasi et al. [45] found that an increase in HE4 precedes an increase in CA-125 by 5 to 8 months and coincides with disease relapse. A larger sample size and a recent retrospective study have confirmed that HE4 can identify relapse before CA125 (126 days on average before clinical confirmation) and that 75% of relapse patients have increased HE4, whereas CA125 is elevated in only 50% of cases [47]. For tracking treatment response and early relapse detection, the combination of CA125 and HE4 may be more helpful than either marker alone [48,49]. In fact, the sensitivity and specificity of the CA-125 and HE4 combination are 76% and 100%, respectively. [50]. In order to improve the information for disease monitoring, Havrilesky et al. (2008) proposed a panel of multiple biomarkers (HE4, MMP7, glycoprotein). Candidates for this panel were selected by the authors using the following standards: (I) overexpression of the encoded protein in ovarian tissue; (II) overexpression of the candidate gene in epithelial ovarian cancer relative to normal ovarian epithelium; (III) localization of the encoded proteins in the extracellular compartment as membrane protein or secreted protein; and (IV) differentiation of ovarian cancer from normal serum using prototypical immunohistochemistry. They found that in 56% of cases, this panel accurately predicted the recurrence of the disease before CA125 elevation, and in 41% of cases, it did so at CA125-equivalent time. Relapse was thus found 6-69 weeks prior to an elevation in CA125 [51]. However, because the specificity of the biomarker panel was not assessed, these findings should be regarded as preliminary and should be scrutinized further. When a disease recurrence is discovered during follow-up, another 2012 study recommends using HE4 in a panel; they selected CA125, HE4, and CA72.4 collectively rather than one at a time [52], based on evidence of elevated serum tumor marker CA72.4 in EOC. The authors claim that combining the two distinct biomarkers, HE4 and CA72.4, performs better and can identify positives in over 75% of patients at follow-up [53]. The findings thus far are very encouraging, but more multicenter randomized trials with larger cohorts are needed to confirm and strengthen the role of HE4 in the recurrence of ovarian cancer.

4. Biomarkers of Interest for the Future

Future biomarkers, like HE4 and CA125, cannot reflect genetic changes in tumors because tumors are heterogeneous and tumor cells are always evolving. The necessity of biomarkers for different forms of cancer has received a lot of attention in the last few years. As a result, liquid biopsy [54,55] was created, a technique for diagnosing illnesses that uses exosomes [55], circulating tumor cells, and circulating extracellular DNA (ecDNA) and RNA of tumor origin. Necrotic or apoptotic cells release ecDNA into the bloodstream, which is present in plasma [54,55]. Mandel and Metais found that ecDNA was present in human blood in 1948 [56]. In 1977, Leon *et al.* found that cancer patients had elevated levels of ecDNA compared to healthy individuals [57]. Later, cancer patients' ecDNA was found to contain cancer-specific DNA that originated from a malignant tumor [55]. This is known as circulating tumor DNA (ctDNA). Somatic genetic changes specific to each tumor are present in cancer, and these changes set cancerous DNA apart from non-cancerous ecDNA [54,55]. The possibility of including all of the tumor's genetic material and any metastases is one of the main benefits of ctDNA [54]. Moreover, the cancer molecular landscape can be repeatedly tested non-invasively with ctDNA. Tissue biopsies that are invasive and painful are not covered by this [54,55]. High levels of agreement between ctDNA and mutational profiles in matched tumors have been reported in the past, especially in breast, colorectal, and non-small cell lung cancers [55]. After surgery, ctDNA has been shown to remain in ovarian cancer patients, which suggests a poor clinical prognosis and higher sensitivity than CA125 [62–58]. The diagnosis and prognosis of breast cancer, prostate cancer, non-small cell lung cancer, and other tumors can all be positively impacted by ctDNA, according to several evidence-based medical research [61–68]. Zhou *et al.* [69] found that ctDNA has a positive value for early detection of ovarian cancer through a systematic review and multivariate analysis. Nevertheless, no meta-analyses regarding the predictive significance of ctDNA in ovarian cancer have been carried out as of yet.

Circulating tumor DNA (ctDNA), a particular type of DNA specific to tumors, has recently been found in patient plasma and has been demonstrated to have a strong correlation with the prognosis of ovarian cancer [70,71,72]. In the identification and diagnosis of ovarian cancer, this novel non-invasive biomarker sets new standards. Recent years have seen notable advancements in our understanding of the role of ctDNA in ovarian cancer and its detection techniques, particularly in the last five years, since the first detection of ctDNA in ovarian cancer in 2012 [73].

5. Brief Overview of ctDNA

In terms of prognostic or diagnostic biomarkers for cancer detection and tracking, ctDNA is starting to show promise. It was initially documented in the 1970s [74] that cancer

patients' blood contained ctDNA. Extracellular DNA (ecDNA) is released into the bloodstream during necrosis, apoptosis, or active tumor release phases. Part of total ctDNA is used to create ecDNA [75], and ctDNA has a half-life in the bloodstream of less than two hours [76]. ctDNA consists of short DNA fragments (150-200 base pairs). The detection of ctDNA is a promising diagnostic tool because of this property as well as its half-life in blood. Comprehensive studies encompassing multiple primary tumor types (e.g., ovarian, bladder, and colorectal cancers) and/or stages have identified 6-log differences in ctDNA content [77,78]. Furthermore, it has been observed that ctDNA is present in over 50% of most cancer types [70] and exhibits a noteworthy association with the molecular pathology of solid tumors [75,79,80]. Furthermore, rather than just displaying a portion of the tumor genome, ctDNA might enable the visualization of the complete genome. Furthermore, serial sample collection is made possible by ctDNA analysis, which is noninvasive for obtaining tumor tissue through biopsy and enables the assessment of quantitative and compositional changes over time. Crucially, analysis of ctDNA has revealed evolutionary adaptation against inhibitors of platinum-based chemotherapy. Non-invasive testing for cancer diagnosis is currently becoming more common due to advancements in technologies for ctDNA analysis and isolation [81,82]. Generally speaking, ctDNA is becoming more and more popular as a clinically viable solution that can represent tumor heterogeneity in both space and time.

6. Methods for Detection of ctDNA

ctDNA analysis was used in a recent prospective study to identify gene mutations in ovarian cancer. Of the patients carrying the mutation, 94% (48/51) had blood samples that were fully consistent with surgical verification and linked to progression-free survival (PFS) [78]. Such high detection efficiency is attributed to the development of genomic analysis technologies. There are currently a number of techniques developed to identify bloodstream ctDNA mutations specific to carcinoma, including PCR-based methods and next-generation sequencing (NGS). ctDNA analysis has been effectively conducted using PCR-based techniques, although these methods are only effective in identifying a restricted set of well-known mutations. Digital PCR (dPCR) and droplet dPCR (ddPCR), two third-generation PCR technologies, have actually been demonstrated to exhibit high specificity (81%) and hypersensitivity (99%) to a known site of ovarian cancer. [77,83,84]. Biological samples of target mutants or wild type can be analyzed using fluorescent probes, and nucleic acid quantification in absolute terms is made possible. In contrast, NGS allows highly sensitive detection of genes in multiple genomic regions in a single assay and is used for DNA mutation profiling and determining the burden of mutations in tumors [85]. Other methods have a wide range of applications, including tumor mutation assessment, burden [86], detection

of epigenetic alterations, and diagnosis or detection of resistance mutations [87,88]. One such method is whole-genome sequencing (WGS). Another is personalized cancer profiling by deep sequencing (CAPP-Seq), which uses NGS to analyze ctDNA in ovarian cancer. Generally speaking, ovarian cancer has shown high diagnostic sensitivity and specificity for ctDNA analysis using a range of techniques.

7. Prediction and Detection of Minimal Residual Ovarian Tumor

Numerous patients have multiple mutations in their tumors after undergoing surgical resection for tumor recurrence, which typically occurs at distant metastatic sites originating from the primary tumor. It is challenging to discern between patients with minimal residual disease and those who have genuinely achieved remission following surgical resection. Research has indicated that ctDNA analysis is a more accurate predictor of treatment response and disease recurrence than imaging or CA-125. It can also predict progression or response to treatment more quickly [89,90,91]. Pereira et al.'s research [90] revealed that residual tumor presence can be identified through the use of customized ctDNA. Additionally, they demonstrated that ctDNA detection had a 7-month prognostic period, which was longer than CT. Parkinson et al. [91] showed that ctDNA in patients with recurrent HGSOC correlated with tumor size at the beginning of treatment in an exploratory analysis using ctDNA as a biomarker to assess treatment response in minimal residual ovarian tumors. Furthermore, they stressed that patients whose ctDNA levels dropped by more than 60% had a noticeably longer time to progression than those whose levels dropped by 60% or less. Furthermore, plasma ctDNA was found by Harris et al. [92] to enable better therapeutic efficacy through somatic chromosomal rearrangements and to monitor cancer patients for recurrence. These findings highlight the potential use of ctDNA mutation detection as an early indicator of recurrence and imply that ctDNA levels are correlated with cancer recurrence in patients.

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