Review Article

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Liquid Biopsy in Ovarian Cancer

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Abstract

Ovarian cancer has always been a perplexing problem to the clinicians. Most cases are detected at an advanced stage with poor and unpredictable response to therapy. Screening of ovarian cancer in general population has not proved to the useful due to low sensitivities of the available tests. There is need for newer tests which can overcome these problems clinicians are facing. Remarkable advances in the field of genetics and molecular techniques have given clinicians a hope for not only early diagnosis but also to monitor response to therapy and detect early recurrences and resistance to drugs. Circulating cell free DNA and circulating tumour cells have given a new hope, though it still hasprove its effectiveness in routine clinical use. This article throws light on these newer developments in ovarian cancer.

Keywords: Circulating Tumour Cells; Circulating Cell Free Tumour DNA; Ovarian Cancer.

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Introduction

Ovarian cancer is an important cause of cancer related death in women. Most of these cancers are diagnosed at an advanced stage of disease where the chances of good outcome are bleak. Even with treatment, morbidity and mortality are significantly high affecting the quality of life due to significant side effects of the treatment. Advanced stage of disease, associated complications with different forms of treatment and the aggressive nature of disease contributes to this burden. Diagnosis of this disease at an early stage where it is still confined to ovaries, may improve the chances of complete treatment and survival, but unfortunately less than 25% are diagnosed early [1]. Over the years there has been advancement is surgical techniques, effective combination chemotherapeutic regimes, and targeted therapy has all contributed significantly to the improvement in survival rates, but in spite of this approximately 70% of these patients expire within 5 years [1,2].

Efforts have been made for screening of cancer ovary, just like cancer of cervix where screening has resulted in significant reductions in incidence and deaths due to cancer cervix. Screening based on CA-125 and sonography has not been found fruitful so as to apply to general population for mass screening. There is need for more specific marker which can detect this disease early. Response of this disease to chemotherapy is not very predictable, markers are also needed to predict the response and resistance to therapy Ovarian tumours are heterogeneous, and changes over time; a subset of tumour cells die whereas some others are reprogramed so as to survive in hostile environment of low tissue oxygenation, cell crowding, poor blood and nutrient availability. Tumour Cells develop the ability to survive and grow these adverse conditions with ability to penetrate blood vessels, they are also able to go to distant places and (metastasis) grow. Cells which metastasize to distant areas may proliferate or remain dormant, which are then resistant to chemotherapy;

whereas those cells who proliferate may not respond to chemotherapeutic drugs adequately [2,21]. Drugs are generally chosen on the basis of clinical trials conducted on large number of population, which may not be equally effective on an individual basis if special tumour biology is not taken care of. Local difference in blood flow and the drug delivery at the target tissue also has a role to play here; this population of tumour cells continues to proliferate. Technology which can guide clinical care of patients at multiple points of time without being invasive is definitely very appealing.

Biology of tumour has aroused much curiosity now, with search for a specific genetic marker/ biomarker which may be used for early diagnosis or recurrence of disease. Circulating Tumour cell and the circulating cell free tumour DNA has attracted much attention, and research in many fields of oncology and other diagnostic fields are focussing on this new marker.Lot of research is being carried on this aspect looking for signature genetic marker for the disease. Cancer cells has many mutations and these tumour specific changes- genomic and epigenomic, may be circulating in the blood as cell free DNA. These tumour specific alterations may be in form of mutation or point mutation, rearranged sequence, microsatellite instability, copy number variation, loss of heterozygosity, DNA methylation or in any other forms. These characteristics can help to differentiate tumour DNA from normal cell free DNA. It can also give patient specific personalised information to detect recurrent or residual disease [20].

The circulating cells of tumour have shown positive correlation with prognostic value in advanced stages cancer. High circulating tumour cells in blood, during treatment have correlated with shorter progression free survival and overall survival.So the cell free DNA and circulating tumour cells in blood can become potential surrogates for the tumour and also called as "Liquid Biopsy". Serial monitoring of a blood samples by practically non-invasive method may be able to pick up the disease earliest for recurrence. This may improve the detection of recurrence with improved sensitivity and specificity and hence better outcome of treatment.

Circulating Cell free Tumour DNA

Presence of significant quantities of tumour DNA was found in cancer patients in 1977 [3]. It is assumed that cell free DNA (cf-DNA) enters plasma when cells at the interphase of proliferation between tumour and circulation lyse or at the time of cell apoptosis. Tumour cell lysis and destruction of micrometastasis also contribute to the cell free tumour DNA. The amount of circulating cell free tumour DNA may be determined by size of tumour, growth rate, destruction, lymphatic and blood circulation [4,5]. Cancer patients may have circulating DNA as high as 1000 ng/ml, average being 180 ng/ml [6]. The size of DNA fragments varies between 0.18 to 21 kilobases, however variations in different samples is common [7].

Uses in Ovarian Cancer

1. Diagnosis:

Elevated levels of cell free-DNA was present in cancer of ovary patients as compared to benign disease, however lack of data in this regard does not tell us if it correlates with size, stage, location. In a meta-analysis of 9 studies by Cheng et al. [8]for evaluating the accuracy of circulating free tumour -DNA for ovarian cancer, 70% sensitivity and 90% specificity for diagnosis was suggested. This makes it a reasonably suitable test as an adjuvant diagnostic tool; the present tools based on CA-125 and human Epididymis protein 4 {HE-4} and USG lacks sufficient sensitivity and specificity acceptable for diagnosis of ovarian cancers [9].

A Tumour specific, Chromosomal instability which is a hallmark of ovarian cancer can be detected in cell free-DNA. Copy number alterations in cell free tumour DNA is also a promising for prediction of ovarian cancer.

An aberrant hypermethylation was observed in early stage ovarian tumours {stage IA/B}; while this hypermethylation of gene panel was not seen in benign and normal ovarian tissue. So a tumour specific hypermethylation in serum is also a promising tool for detection.

Its use for early detection of ovarian cancer is the future utility of this test, though challenging. Technical hurdles and standardisation of plasma processing and analysis needs to be overcome for this development to take further.

2. Assessing Response to Treatment

Patients of epithelial ovarian cancer have higher quantities of circulating cell free tumour DNA as well as mitochondrial DNA. Cell free tumour DNA could differentiate between patients of pre & post chemotherapy ovarian cancer which may be used to asses for monitoring response to chemotherapy [10]. Mutations are common and Mutant DNA after surgical treatment may be useful for follow up of these patients where chemotherapy is not used. Mutations in p53 were also identified. Hypermethylation of RASSF1A (tumour suppressor gene) was found in cell

free-DNA of ovarian cancer patients, which were higher in stages III & IV of disease. Cancer progression was co-related with presence of KRAS mutations, and P53 antibody [22,23].

Resistance to chemotherapy in ovarian cancer is challenging; Cisplatin, Carboplatin and Paclitaxel is the mainstay chemotherapy for advanced stage patient, acquired resistance to these first line agents is challenging. Truncated mutation are increased in these group of patients who show resistance. Exome wide analysis of cell free tumour DNA may become an adjunct approach for detecting mutations in drug resistance advanced Ovarian cancer patients.

BRCA1/2 reversion mutations can be detected by cell free DNA sequencing analysis in ovarian and breast cancer patients [25].

Analysis of circulating tumour requires very sensitive methods because of small percentage of tumour specific DNA intermingled with other native DNA and hence is challenging and requires highly sensitive techniques. Classical methods analyzing cell-free DNA include quantitative real-time polymerase chain reaction (PCR)-based, fluorescence-based, and spectrophotometric approaches. More recently, a variety of digital genomic methods have been developed to improve identification of generic alterations in circulating tumour DNA. Digital PCR has now emerged as a sensitive tool to detect point mutations in circulating tumour DNA at low allele fractions, which comprises droplet-based systems, microfluidic platforms for parallel PCR [10], and an approach called BEAMing (beads, emulsions, amplification and magnetics). Next generation sequencing technologies are currently being applied to plasma DNA analysis. These high-throughput, low-cost, sequencing technologies identify widespread circulating tumour DNA alterations across wide genomic regions. [PCR based- nested, Mutant allele-specific PCR, Mass spectrometry, Bi-PAP-A amplification etc.], digital PCR [droplet based PCR, microfluidic based, etc.] targeted deep sequencing, Whole-genome sequencing [Digital karyotyping, PARE].

Cancer harbours somatic genetic mutations and these tumour-specific alterations can be detected in circulating tumour DNA. Therefore, it carries genomic and epigenomic alterations concordant to the tumour mutational spectrum, such as point mutations, degree of integrity, rearranged genomic sequences, copy number variation (CNV), microsatellite instability (MSI), loss of heterozygosity (LOH), and DNA methylation. These biological characteristics discriminate circulating tumour DNA from normal cell-free DNA and assure circulating tumour DNA as a specific biomarker that provides personalized information to detect residual disease or monitor tumour progression during therapy [26].

Circulating Tumour Cells

Primary tumour sites may release cells in circulation [11], their levels correlate with therapeutic response and survival. These circulating tumour cells have been considered as "Liquid biopsy" for metastatic tumours. Circulating tumour cells can be detected by –cell surface marker dependant and independent methods. Epithelial cell adhesion molecule (EpCAM)is the most commonly used antigen present on surface and is absent in normal white blood cells [12], technology which uses this antigen (EpCAM) for detection of circulating tumour cells has been well accepted. The use of cell search is presently limited to few cancers egmetastatic breast cancer, prostate cancer, colorectal cancer. The cell-search method for ovarian cancer do not correlate well with clinical features and outcome with the presently used technique. Marker independent methods, which does not relies on one antigen, but on biophysical properties eg size, deformability etc. are still evolving. There is unmet need of methods to evaluate circulating tumour cells as biomarkers continue.

Utility

1. For Diagnosis

Lot of research is going on for isolation of circulating tumour cells [13,14]; however the presence of circulating tumour cells is associated with adverse and elevated CA-125 levels & HE-4. This increased number of circulating tumour cells also correlated with adverse progression free survival and overall survival. Various genes could act as potential biomarker KRT7, WT1, EPCAM, MUC16, MUC1, KRT 18, KRT 19 etc. to name a few [13]. These technologies need to be more sensitive as well as specific for early detection of ovarian cancer and need further development.

2. Identification of Resistance Mutation

Circulating tumour cells can be used for isolating tumour cells as well as to monitor mutations related to drug resistance in ovarian cancersover time. Epithelial marker- EpCAM was found markedly low with only 8% of baseline and 4% of follow-up in circulating tumour cells (CTCs) positive samples

[15]. PPIC gene in Circulating Tumour Cells was significantly overexpressed in baseline and follow up groups [13], it belongs to cyclopilins that have peptidyl-prolylcistransisomerase (PPIase), an intracellular receptors for immune-suppressive drug cyclosporine A (PPIA)[16], Research has shown PPICpositive cancer cells are more likely found in cancer cells [15]. Few more genes - MRP1–10, MDR1, ERCC1, RRM1, RRM2 have shown promising results for resistance tochemotherapy [13].

The Added Worth of Cell free Tumour DNA and Circulating Tumour Cells in the Diagnosis of Ovarian Cancer

CA-125 is still the main marker in ovarian cancer detection in the clinical setting, and is also used for monitoring therapeutic effects; but CA 125 is raised in many other benign and physiological conditions. Diagnostic specificity of CA-125 alone is not optimum. Another marker HE4 has emerged as a new biomarker for both diagnosis and monitoring response, but even this marker is increased in many benign conditions eg fibroids, polyps of uterine cavity, endometriosis, ovarian cysts. However their expression in ovarian cancer is different [17]. When used together diagnostic accuracy may be better. Another parameter which has come up as a diagnostic value is RMI [risk of malignancy index] along with CA125 where ROC curve was generated to predict malignancy [18]. Vanderstichele et al found that sensitivity of cell free-DNA was 2 to 5 times more than CA 125. International Ovarian Tumour Analysis group has started study on cell free DNA tests integration for predicting this cancer.

Heterogeneity of primary tumour often hampers detection of circulating tumour cells, no specific marker is expressed in all tumour types. There is need for more specific markers that will help in early diagnosis of this cancer. Presence of circulating tumour cells was often associated with shorter survival in ovarian cancers so may be used as a prognostic marker and hence for treatment monitoring. Molecular characteristics of cell free tumour DNA and circulating tumour cells can be detected in plasma/ blood of cancer patients. Cell free- DNA is easier to isolate and can be used for treatment monitoring. Circulating tumour cells help in detection of tumour mutations and heterogeneity. It has a role in clinical management also. If both are assessed it may become a source of tumour tissue giving information on transcriptome and genome [19]. Cell free DNA and circulating tumour cells can also be used in guiding specific targeted therapy by detecting multiple changes of gene expression, avoiding the harmful effects [24].

Conclusion

The technique of isolating cell free tumour -DNA and circulating tumour cells hold promise for future advancements in ovarian cancer approach and management. It may represent a turning point as liquid biopsy, which can be obtained at multiple point of time. Mutational profiling of these cell free tumour DNA and circulating tumour cells may help in early assessment of treatment response and enable early diagnosis also. Future advancement on molecular characterisation of cell free-DNA and circulating tumour cells will help in detecting drug resistance early and establish more personalised therapy cost effectively with more accurate risk stratification and prognosis. Even though many standardisation and details are yet to be developed, cell freetumour DNA and circulating tumour cells hold lot of scope for future emerging techniques for early detection, accurate diagnosis, targeted and personalised therapy and improved monitoring and prognostication in ovarian cancer.Significant progress has been accomplished so far and still more work needs to be done to optimize the best clinical use of this applicationand clinicians need to be aware of this new emerging technology for early detection and treatment monitoring for ovarian cancer patients.

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