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Opinion

Can Liquid Biopsy Cancer Research Offering Personalized Cancer Treatment in Gynecology be a Realistic Expectation?

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Abstract

This paper encourages understanding a non-invasive technology—the "Liquid biopsy", including circulating tumor cells (CTCs) and circulating tumor DNA (ct DNA) technology, that can help diagnose early cancers, cancer relapse, and resistance to chemotherapy. This paper reviews various CTCs and ct DNA studies in the literature about their applications in gynecological cancers. Hopefully, further translational cancer research in gynecology will enable personalized cancer treatment to become a realistic expectation.

Keywords: gynecological cancers; liquid biopsy; circulating tumor cells; circulating tumor DNA

1. Introduction

Gynecological cancers, including uterine, ovarian, and cervical cancer, negatively impact women's lives and suffering. Ovarian cancer is the leading cause of gynecological cancer death and is diagnosed at advanced stage III/IV. Despite new technological development, the cancer management of gynecological cancer does not have major changes compared with two decades ago. Computed tomography (CT), magnetic resonance imaging (MRI), and positron emission tomography and computed tomography (PET-CT) remain only accurately diagnostic when gynecological cancers reach certain sizes and produce symptoms of pain, bleeding, or mass arising from the pelvis. Advancing technology such as robotic surgery might have improved surgical excision of cancers and improved the sensitivity and accuracy in imaging diagnosis. However, many women still suffer from late-stage cancers, resistance to chemotherapy, and failure to detect early relapse of cancers. The chemotherapy used often is a standard chemotherapeutic medication to start with. They need changes to alternative drugs when drug resistance is noted or a cancer relapse after chemotherapy. Over the years, new translational research developments in gynecological cancer management must be reviewed to become new armaments for gynecological cancer treatment.

Compared to the traditional treatment based on the pathology of solid tumors, tumor markers, and imaging or surgical staging, the recent development of using liquid biopsy, information derived from circulating tumor cells (CTCs), circulating tumor DNA (ct DNA), and their potential applications can largely enhance the cancer diagnosis in its early stage, metastasis in early stage cancers, cancer chemoresistance and relapse after treatment. Hopefully, new translational research can identify more biomarkers with high sensitivity and specificity, thus enabling early diagnosis and development of personalized, targeted chemotherapy treatment.

2. What is "Liquid biopsy"?

Technology advancements have enabled the collection and identification of circulating tumor cells (CTCs) and circulating genetic materials from patients' body fluids, which include blood, urine, peritoneal fluid, and lavage. It leads to new fields of biospecimens in cancer research. Cancer cells and abundant circulating genetics can also be collected from the circulation. Thus, the blood liquid biopsy with circulating tumor cells and ct DNA is the primarily evaluated biomarker. They offer abundant information for researchers and clinicians to develop tools for early cancer diagnosis, staging, and response to treatment.

2.1 Circulating Tumor Cells

When cancer forms, cancer cells can extravasate into the "neo-vessels" or transform into endothelial cells, making it easier to disseminate into the human circulation as circulating tumor cells. Circulating tumor cells (CTCs) are cancer cells shed into the peripheral blood circulation from primary or metastatic tumors. Because the phenotypic and genetic signatures of these rare cells can provide important information for cancer staging and treatment, further research on their characteristics and properties is an exciting area.

Many authors have also developed various microfluidic devices such as the CellSearch® system (Menarini Silicon Biosystems, Inc), the ISET® technology (Rarecells, Inc), the PARSORTIX system (Angle), and the MetaCell®,

ScreenCell® technology etc. Based on the isolation of cells by immunoaffinity, size or density based, membrane dielectric capacitance, and combined methods, these microfluidic devices isolate an adequate number of CTCs. The evaluation of CTC counts and CTC cluster abundance can significantly improve the assessment of cancer burden in cancer patients undergoing therapy. Poveda *et al*. [1], in their study, demonstrated that increased numbers of CTCs indicated an unfavorable prognosis for progression-free survival and overall survival in ovarian cancer patients. In another group of 71 patients with ovarian cancer, the role of CTC counts was studied for various disease stages. Fan *et al*. [2] found that 43/71 (60.6%) patients had detectable CTCs, including 1/10 (10%) early-stage, 39/52 (73.1%) late-stage, and 3/4 (75%) unstaged patients. CTCs at diagnosis seem to correlate with adverse clinicopathological features and worse clinical outcomes in ovarian cancer patients when associated with elevated cancer antigen 125 (CA-125) and Human Epididymis Protein 4 (HE4) levels [3,4]. In another study of 118 patients diagnosed with ovarian cancer, Kolostova *et al*. [5] could successfully isolate CTCs in 77 patients (65.2%), which correlated with the proliferation potential in different histology ovarian cancer subtypes. Similarly, in advanced endometrial cancer, CTCs could be demonstrated in high-risk cancer patients [6].

Given the clinical significance of CTCs in this translational cancer research, Tumour Cells (TCs) have been incorporated into the fifth edition of the World Health Organization (WHO) Classification of Tumours: Breast Tumours and the seventh edition of the American Joint Committee on Cancer (AJCC) Staging Manual. The term 'cM0 (i+)'. indicates no overt metastasis, but the blood has detected tumor cells. This indicates no overt metastasis, but the blood has detected tumor cells. Nevertheless, CTCs have yet to be adopted into the clinical practice guidelines of major cancer societies.

Circulating tumor cells (CTCs) are the sources for non-invasive and dynamic cancer profiling with many of the same benefits as circulating tumor DNA profiling The phenotypic study of single-cell isolation from tumor cell clusters would enable DNA sequencing and in-depth mutation studies, allowing DNA biomarkers to be developed. The development of DNA sequencing will also enhance a better understanding of the cancer formation, recurrence, and chemoresistance of cancer.

2.1.1 CTC Enrichment

To identify rare CTCs, researchers have recently developed an ultrahigh-throughput size-based separation method for separating CTC and other disease cells from blood [7–10]. Due to the straightforward manufacturing protocol and higher throughput, 4-log white blood cell (WBC) depletion can be achieved. The method exploits the different focal positions of larger CTCs versus smaller blood cells due to the microfluidic device's combination of inertial and Dean drag forces, enabling rapid and continuous isolation of viable CTCs. The device's simplicity, robustness, and high throughput ensure the feasibility of using this method in a clinical setting. As a result, the technique has few technical limitations and provides excellent recovery, viability, and WBC depletion.

2.1.2 CTC-Derived Models

In the past, cancer cell cultures from primary cancers relied on long-term maintenance of cancer cells from primary cancers, and then cultured in a research laboratory to develop into cancer cell lines [11]. This process usually takes >6 months to a year with an efficiency of 10 to 20% under various influencing factors [12]. Dr. Khoo and her research team [13] recently adopted a simple, unique microfluidic-based culture device and reported obtaining CTC culture with minimal pre-processing and does not require prior enrichment and the use of growth factors supplements. This new label-free analytical tool based on liquid biopsy patient-derived tumor models have been validated in over 300 samples, allowing for early disease outcome prediction. They used laser-ablated microwells to harbor cancer cell culture with blood leucocytes as a co-culture, thus mimicking the tumor microenvironment *in vivo* [13–16]. With these culture devices, they had successfully estimated CTC clusters within two weeks with an overall successful yield of about 50%, depending on the sample cohort. The proportion of viable cell clusters within microwells collected by the device can be subjected to drug screening to assess the cancer response to chemotherapy. During chemotherapy, the cluster-forming frequency of CTCs diminished; this might reflect the patient's *in vivo* response to treatment [16]. Simple cell or cancer cell clusters can allow molecular characterization of cancer and the study of its genotypes and aberrant genes.

Tumor models of heterogeneous clusters of circulating tumor and immune cells unique to each patient can be established in about one treatment cycle (14 days), allowing clinicians to intervene quickly based on readouts. The four core parameters used to analyze the LIQBP platform are size, roughness, and thickness per unit area (TA). The phenotypes clustered in pretreated patient samples (n = 4) differ from those established in healthy controls (n = 5). LIQBP can also distinguish response subtypes (e.g., treatment time point, tumor type).

2.2 Circulating Tumor DNA (ct DNA) or Cell-Free DNA (cf-DNA)

During normal cell death, DNA fragments are constantly released into the blood vessels but rapidly removed by the body via the liver, kidney, and spleen. Thus, the DNA levels are very low. However, this clearance process is inefficient for malignancy cells, leading to high circulating tumor DNA in the patient's blood. Cancers often detected late, including the pancreas and ovarian, have high ct

DNA levels. Therefore, it could allow the detection of typically late-stage malignancies. A study of ct DNA showed it was found in more than 75% of known cancer patients, and there was a quantitative correlation between the amount of ctDNA and a patient's tumor burden [17]; Therefore, ct DNA might correspond to the diagnosis of late-stage cancers.

Liggett *et al*. [18], in their study, found that methylation patterns of ct DNA can differentiate between benign and malignant tumors. In his study of 30 patients with ovarian cancer compared with healthy controls, methylated tumor DNA levels of patients were higher, with a sensitivity of 90% and specificity of 86.7% [18]. Martignetti *et al*. [19] had shown that ct DNAs were detectible radiologically- and biopsy-proven relapsed ovarian cancer patients, whereas the pre-treatment positive CA-125 levels did not show elevation. Therefore, these findings suggest that screening methylation ct DNA in ovarian cancer will support it as a better biomarker than CA-125. Similarly applied to other cancers, Diehl *et al*. [20] reported that detecting ct DNA mutants in colon cancer patients after surgery and chemotherapy was more clinically useful than the conventional tumor markers—cancer embryonic antigen (CEA). Nevertheless, the technique to detect the total amount and integrity of ct DNA is still exciting because it may allow to detect specific genetic biomarkers based on DNA mutations, DNA methylation, DNA/RNA expression sensitive and specific in gynaecological tumors.

2.3 Circulating Tumor RNA Exosomes, and Bio-Proteins

Other circulating genetic materials such as miRNA, vesicles, and proteins are being studied. There is increasing evidence that miRNAs can be used as biomarkers to detect early diagnosis and predict survival and response to treatment [21]. Recent studies on miRNAs showed that in ovarian cancer, miRNAs participate in developing drug resistance. While in endometrial cancer, they play important roles in tumor formation, including cell proliferation, migration, and metastasis. miRNA methylation has also been studied for its tissue profile and serum expression. However, few studies have been conducted and concluded that miRNA in gynecological cancer is a useful biomarker.

Besides the protein CA-125, serum HE4 level is a reliable biomarker for managing ovarian and endometrial cancer patients [22].

Some studies on endometrial carcinoma and metastasis found that the expression of single specific genetic biomarkers such as Migration-inducing gene 7 (Mig7), Cytokeratin 19 (CK19), and thyroid transcription factor-1 (TTF-1) correlated with the Cancer staging system - tumor, lymph node and metastasis (TNM), vascular infiltration, and lymph node metastasis and reduced median survival time. Thus, these new genetic biomarkers are proposed as good prognostic predictors for endometrial cancer and metastasis [23]. Zhang *et al*. [24], in their study of endometrial carcinoma, found that circulating tumor cells that were positive for thyroid transcription factor-1 (TTF-1) could predict recurrence and correlate with survival rates.

Since there is no requirement for the enrichment and good culture conditions, it is more convenient to use cf-DNA as a biomarker than CTCs. The detected gene panels in cf-DNA were also used as bedside tests to monitor treatment response with reliability and efficiency.

2.4 Other Cancer Cells from Liquid Biopsies

A recent study used inertial microfluidics to separate exfoliated bladder cancer cells (EBCCs) from bladder-flush urine [25]. Previously, such techniques were optimized for detecting circulating tumor cells (CTCs) from blood-based liquid biopsies [26]. Following a pre-treatment step by filtration to remove larger impurities such as squamous epithelial cells (30–60 m), the microfluidic device concentrates and processes the sample at a rapid rate of 1.7 mL/min to isolate target EBCCs (11–15 m). The device can be multiplexed, enriching a 50 mL sample in less than 10 minutes. The device had a high sensitivity (93.3 ± 4.8%), and the cells collected were still alive.

3. Discussion

Although the CA-125 and transvaginal ultrasound are the primary investigations to diagnose genital cancers, they lack both sensitivity and specificity for early cancer detection. The recent development in studying CTCs and ct DNA from patients' circulation is exciting. A blood test becomes a "liquid" biopsy for cancer, providing information on a patient's circulation for early cancer diagnosis, staging information, and tracking for cancer chemoresistance or recurrence. The number of CTCs was very low in peripheral blood. Thus, many existing *in vitro* and *in vivo* cell culture systems are too inefficient to yield enough tumor cells for reliable and functional analyses. With new technology development, these innovative microfluidic systems will now provide a unique diagnostic medical device for functional studies of viable CTCs. The metastatic potential of CTCs was verified by injection into the immunodeficient mice. Therefore, the subsequent development of either cancer cell lines or xenografts from CTCs can provide important functional insights into the profile of these cancers. Although these studies are promising, there are limited advances in liquid biopsy in gynecological cancers compared to other tumors such as colorectal, breast, or prostate.

In the past, it was due to a lack of methods to study CTCs that had led to cancer relapse due to distant metastasis and failure of chemotherapy. The microfluidic culture system in the laboratory can be a new innovative device for the diagnostic and therapeutic management of cancers, as described in this paper. This microfluidic culture device to study CTCs, implemented as liquid biopsy, can supplement cancer treatment using traditional surgical and imaging staging, tissue pathology, and tumor mark-

ers. This research tool can provide an objective, effective, rapid, and affordable tool for managing cancer patients. Besides, the findings of CTCs, which are probably an important source of distant metastasis, will be interesting. The subsequent development of cancer cell lines and cancer xenografts from CTCs could represent a great opportunity to decode metastatic cancer events and test cancer drugs targeting CTCs *in vitro* and *in vivo*.

The minimally invasive nature of blood taking, instead of surgical biopsies, could allow a more frequent follow-up assessment of the clinical treatment and allow early detection of cancer or recurrence. The future of CTCs and ct DNA/ct RNA research is challenging because they can lead to the development of molecular targets for treatment, detection, and monitoring. Areas of improvement are needed to further enhance the clinical usefulness of these research tools, for example:

(1) A better understanding of cancer clonality and its relationship with CTCs. In the future, we must confirm whether CTCs are the most aggressive clones of primary cancer and are relevant to metastasis, chemoresistance, and relapse, thus influencing our initial treatment decision.

(2) Multiple tumor heterogeneity of gynecological cancers could lead to an incomplete picture of the mutational profile of the cancers we studied. This information could be crucial for planning targeted therapy for our patients.

(3) The lack of standardized analytical techniques to identify different genetic profiles can lead to the identification of many gene biomarkers, but none is sensitive and specific enough for clinical use. Developing a new, rapid, low-cost standard DNA/RNA biomarker is necessary.

(4) Increasing the yield of the microfluidic culture system is essential to ensure adequate CTC information can be obtained from each patient we treat.

(5) No highly sensitive and specific biomarkers and targeted therapies for patients with gynecological cancers are used for their treatment. Because liquid biopsy may offer new perspective tools for monitoring tumor development, effective treatment, and the occurrence of treatment resistance, it is of prime importance to explore the development of liquid biopsy in gynecological cancer.

4. Conclusions

Research on circulating tumor cells or ct DNA research will allow this non-invasive technology to become a common application in cancer management. They are also valuable sources of genetic materials for the molecular analysis of cancer in their formation and diagnosis. With increasing studies on gynecological tumors, oncologists will have increasing armaments to provide personalized patient treatment. We envisage when CTCs and ct DNA may become a bedside procedure that can assist in patient assessment of cancer staging and therapeutic decision-making by timely identification of aggressive cancer subclones, chemoresistance, and early detection of metastasis, which overcomes the limitation of this treatment. Even though these blood liquid biopsy data, based on the above research reports, offer a realistic expectation for personalized cancer management in Gynaecology, they are considered only experimental approaches, and none of them has reached clinical application in gynecological cancer treatment. Finally, it is hopeful that gynecological oncologists are aware of this updated laboratory research of CTCs and ct DNA and will soon adopt them in our clinical practice.

Author Contributions

FW performed the search and contributed the information and writing of the paper. BLK and KKLC contributed the conceptualization of the paper, advising the manuscript's writing and editorial changes. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work to take public responsibility for appropriate portions of the content and agreed to be accountable for all aspects of the work in ensuring that questions related to its accuracy or integrity.

Ethics Approval and Consent to Participate

Not applicable.

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Conflict of Interest

The authors declare no conflict of interest. Felix Wong is employed by Women and Babies Clinic, and the Clinic did not participate in the preparation and publication of the article.

Felix Wong is serving as one of the Editorial Board members of this journal. We declare that Felix Wong had no involvement in the peer review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Luca Roncati.

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