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Review Article

Liquid Biopsy: Promising means in the Fight against Cancer

J.Chouef *[1], H.Medyouni[2], O.Siyouri[3], C.Chbihi[4], S.Mhirech[5], L.Amaadour[6], K.Oualla[7], Z.Benbrahim[7], S.Arifi[8], N.Mellas[9]

1,2,3,4,6,7,8,9. Medical Oncology Department, chu hassan ii, fes

5. Radiotherapy Department, chu hassan ii, fes

*__Correspondence to__: Jihane Chouef, Medical Oncology Department, chu hassan ii, fes.

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ABSTRACT

A traditional biopsy performed on a tissue sample presents risks and some challenges. The target area may be difficult to reach and bleeding as well as pain may be felt up to a month after the procedure. Patients can expect high medical costs and must wait up to four weeks for their results, an obvious problem when a patient has aggressive cancer. Over the past two decades, scientists have worked to develop an alternative: liquid biopsies. Like ordinary biopsies, these can detect cancers, but to carry them out, it is enough to take bodily fluids, generally blood. The advantage of blood over tissue samples is that it can be collected easily and repeatedly. According to Jeffrey Campbell Thompson, senior lecturer in medicine at the Perelman School of Medicine, a standard sample of 7.5 to 10 mL of "peripheral blood," usually from a patient's arm, is sufficient. suffering from cancer, and to wait a short week for the results to arrive. This reduced time can help speed up the processing schedule. However, specialists say that research to develop more effective liquid biopsies is progressing rapidly, and they are hopeful that this tool will soon make it possible to identify cancers, at any stage.

***Keywords**: Liquid biopsy –cancer –prevention -Surveillance*

Introduction

A liquid biopsy , also called a fluid biopsy, is a blood test to look for cancer cells or fragments of tumor DNA circulating in the blood. It can also be more rarely carried out on urine or saliva samples. Historically, Mandel and Metais were the first to describe in 1948 the presence of DNA in the bloodstream of humans outside of cells, which they called cell-free DNA (ctDNA) (1). Nearly 30 years later, Leon et al. found significantly higher levels of this type of DNA in the serum of patients with metastatic cancer , which decreased after response to treatment (2). Then in 1989, Stroun et al. (3) reported that a fraction of ctDNA is derived from cancer cells , so it is called circulating tumor DNA (ctDNA). ctDNA is a short fragment (100 bp) mainly released by cells located in the tumor bed (4-5). With the successful sequencing of the human genome followed by the emergence of new sequencing technologies, cDNA detection and sequencing have become readily available in the real-world setting, becoming a promising non-invasive technique frequently used in modern oncology.

Principle of Liquid Biopsy

A tumor often releases some of its DNA (probably coming from apoptotic tumor cells) into the bloodstream as well as a few tumor cells. The analysis of tumor DNA, directly from plasma or after isolation of CTCs, can provide information on the nature of mutations present in the tumor and therefore guide the choice of treatment, without the need for a biopsy. of tissue which is often delicate, particularly for lung cancer. Although the quantities of tumor DNA are low (of the order of a few ng per ml in general), and normal cellular DNA originating from lymphocytes is often in the majority, the precision of the data obtained by next generation sequencing (NGS) makes it possible to identify mutations present in tumor DNA (6). Such a "liquid biopsy" clearly has many advantages, especially since it can be repeated during treatment to monitor the evolution of the tumor genome.

The steps in liquid biopsy include blood collection, sample processing, and DNA isolation . Each of these steps can affect the final stability of the cDNA , highlighting the need for standardized procedures. Plasma is more suitable than serum since the latter contains much more cDNA from nonmalignant cells (7) . Therefore, when collecting blood, it is crucial to use tubes containing anticoagulants that do not interfere with the stability of the cDNA. Heparin is contraindicated because it does not restrict cDNA-degrading endonuclease activity and could potentially inhibit the polymerase chain reaction (PCR) that is sometimes used to amplify cDNA (8) .

Special collection tubes have been developed containing fixing agents for leukocyte stabilization and preservation of cDNA integrity, such as PAXgene™ tubes (Qiagen, Germany), DNA™ cell-free blood collection tubes (Streck Inc., Omaha, USA) and cDNA collection tubes. (Roche Diagnostics, Germany). However, EDTA-coated tubes could also be used as they impart good stability to the cDNA, but plasma isolation should be performed on the same day of collection to limit the release of leukocyte-derived DNA .

What areas of application?

The analysis of circulating tumor DNA then makes it possible to regularly monitor the evolution of tumor cells, to see the reduction in the number of those carrying the targeted anomaly, the rise in power of initially minority species and, possibly, the appearance of new mutations. It is a valuable tool which should make it possible to know when the targeted treatment risks becoming ineffective and which new approach should then be considered. The real possibility of implementing such a process on a large scale was the subject of a

recent article (9), and its use in clinical research has also been reported to evaluate the appearance of resistance (10) or for the early detection of metastases (11). Here too, it remains to carry out more extensive validation studies, to demonstrate real clinical utility, but we can clearly see that this approach should facilitate the early detection of metastases, the rational modification of therapy, and help to avoid overtreatment. by an agent who is no longer effective. These repeated tests only require a simple blood sample, and are perhaps more revealing about the dynamics of the tumor than a tumor biopsy which would give a fixed image. Analysis of circulating DNA can also be carried out after removal of the tumor, to check the absence of tumor DNA which indicates complete resection. All this needs to be clarified, but we can bet that this monitoring will become more and more common.

Several uses in Clinical Practice

In localized situation: One of the essential objectives of ctDNA in plasma is the identification of minimal residual disease (MRD) in cancer patients at risk of disease relapse after the curative approach. Several articles have addressed this question to detect the feasibility of such an innovative approach aimed at limiting, on the one hand, the unnecessary administration of adjuvant treatment in people with a low risk of disease relapse, but also to identify the powerful beneficiaries of such an approach. In several solid tumor subtypes such as breast, lung, and colorectal cancers, ctDNA has been used to detect MRDs using patient-specific techniques (12-13-14).

In a metastatic situation : The progression of metastatic cancer is a complex and evolving process marked by the emergence of different subclones, which contributes to tumor genomic heterogeneity . Tissue genomic profiling, while of extreme importance in precision oncology , is unable to capture spatial and temporal heterogeneity, thus potentially missing important targetable factors (15) . On the other hand, liquid biopsy is a non-invasive and easily reproducible method that allows the exploration of the spatial and temporal evolution of the cancer genome, as shown by different studies that have compared it to tissue analysis (16 - 17-18) .

Liquid biopsy could also be used to monitor early tumor response. For example, Dawson et al. reported data from 30 women with breast cancer, followed by radiological imaging, approximately 15–3, and ctDNA. The authors showed that ctDNA is a useful, specific, and highly sensitive biomarker for metastatic breast cancer , even superior to other biomarkers (19) . Additionally, Page et al. reported 9 cases of patients with metastatic

breast cancer, in whom ctDNA variations were prognostic, useful in detecting new mutations that may alter the treatment plan, as well as useful in assessing treatment response during of monitoring disease status, while approximately 15-3 did not. Match variation (20) . Another illustration of the use of liquid biopsies for follow-up is presented in the data collected from serial blood samples from the TOPARP-A trial reported by Goodall et al. Where a decrease in ctDNA level after 4 weeks and later after 8 weeks correlated with longer progression-free survival and longer overall survival, respectively (21).

With new specific targeted therapies, new resistant mutations appear and it therefore appears necessary to discover them and try to target them. The T790M mutation is the main example, because 50% of patients with mNSCLC, an EGFR mutant disease, acquire this mutation after the use of a first-line TKI, and a third-generation TKI can therefore be used instead of switching to chemotherapy with a favorable response rate and PFS (22-23) . In the TOPARP-A trial, the clinical value of liquid biopsies in detecting genetic composition , primary mutations and emerging subclones is demonstrated, where in blood samples from patients with HRD or LOH mutations, which responded to olaparib , the allele frequency fell to less than 5%, while it remained stable in the patient who did not respond. Additionally, in patients for whom no response was observed, two ATM frameshift mutations were identified after treatment (21).

Accurate interpretation of ctDNA analysis remains a challenge for scientists because many variable genomic mutations could be found, without correlation or real clinical benefit (24) .

An example of randomly discovered mutations is illustrated in a Chinese randomized trial in which approximately 6,200 mNSCLC patients were tested, and 1% of them had gBRCAm. This study showed a positive correlation between gBRCA mutation and early onset of NSCLC (25). But how to apply this in the real clinical setting? Additionally, should patients with a gBRCA mutation be screened for lung cancer? Questions that remain unanswered. Slavin et al. reported data from an observational case series on more than 50 cancer types , with more than 10,000 patients, regarding the prevalence of germline mutations accidental in patients with advanced solid tumors and, as expected, several mutations were found without clear guidelines for counseling, screening, or possible therapeutic guidance (26) .

Several limitations of ctDNA analysis remain major obstacles in real-world practice. First, the sensitivity of ctDNA analysis, which can result in a high rate of false negative results, as well as its specificity, as it can be affected by the release of ctDNA from non-cancerous cells. Second, variability in cDNA release from tumor cells can significantly affect results. Third, financial and ethical limitations may also affect the availability of this innovative approach in diagnostic and therapeutic approaches. Finally, interpretation of

ctDNA results can be difficult, as not all mutations are clinically relevant (27-28).

Outlook

In recent years, blood-derived liquid biopsy has emerged as a potential alternative to tumor tissue samples in various indications. However, many other bodily sources besides blood are being evaluated to replace or complement this approach.

Detection of ctDNA in saliva is an alternative method, mainly in patients with head and neck tumors . This approach has several advantages: a non-invasive screening tool, real-time monitoring of HNC patients as well as the ideal site to reflect molecular, genomic and pathological alterations in the head and neck region. However, the presence of numerous nontumorigenic components in the medium as well as the presence of different contributors in saliva make ctDNA analysis and identification extremely difficult (29) . This source of cDNA in saliva may provide an option for the detection of MRDs in oral cancers after chemoradiation .

Urine can also serve as an interesting source of cDNA from urological cancers , divided into 2 types: trtDNA (transrenal tumor DNA), originating from plasma, and the second originating from tumor excretion directly in urine. . This is a non-invasive technique that does not require the intervention of health professionals. However, there are several obstacles, including the dilution effect on DNA in large volumes of urine, but also conservation and transport maneuvers with the short half-life of unpreserved DNA (30-31). Several trials have evaluated the role of trtDNA as a tool for detecting molecular alterations such as EGFR in NSCLC, but have also served as a disease monitoring tool in cancer patients. early breast cancer after surgery and hepatocellular carcinoma after tumor removal (32-33). On the other hand, cell-free urinary DNA , originating directly from prostate, bladder and kidney carcinoma, can be used to monitor disease relapses. Zhang et al. found that in urine samples there is a higher identification of genomic alterations associated with urothelial carcinoma compared to plasma with better concordance with tumor samples but also ease of collection and storage of urine samples. urine in storage containers. Malignant pleural and peritoneal fluids , involving tumor cells infiltrating their mucosa, may be enriched in nonhematopoietic ctDNA cells even if cytology was negative. The advantage is that samples are easily acquired via pleural sampling with data demonstrating that more conductive alterations have been detected in pleural fluid compared to plasma, but they also involve invasive procedures (31). In CSF , cDNA is much more abundant than in the plasma of patients with glioma or meningeal involvement. Despite their potential role as a diagnostic tool for individuals with equivocal

histology or to monitor response or predict disease relapse in CNS tumors, their implementation in clinical practice can be difficult due to the invasive nature of the acquisition but also the small size of the samples which can limit the analysis of ctDNA (31).

Other difficult sources of ctDNA are currently being studied, such as seminal fluid, sputum, stool, and uterine washing.

Conclusion

ctDNA analysis is an important tool in the area of precision and personalized oncology. It is a non-invasive and easily reproducible way to detect alterations in susceptibility and resistance. The study of ctDNA kinetics, despite its complexity, could be used to adapt therapeutic strategies over time, but could also become an effective indicator of therapeutic efficacy.

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