Review Article

Liquid biopsy and its significance in tumour – Detection in the field of pathology

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Abstract

The treatment of cancer has remarkably improved because of increased knowledge of the abnormalities at the molecular level, which results in human cancer growth. This has initiated the development of ever more successful as well as effective targeted cancer therapies. Detection of cancer is diagnosed basically by performing routine biopsy/cytology, which has many drawbacks. Therefore, the concept of liquid biopsy has been introduced to oncology, which has the potential to revolutionise the management of cancer patients, eliminating the invasive procedures needed to obtain tissue samples and provide information. Liquid biopsy is the analysis of tumour cells or tumour cell products obtained from blood or other body fluids, providing a broad range of opportunities in the field of pathology. Here, we focus on the most prominent liquid biopsy markers, circulating tumour cells and circulating tumour-derived deoxyribonucleic acid (DNA), in the blood of patients. In this review, we discuss recent clinical studies on these biomarkers for early detection and prognostication of cancer, which helps in successful management. Hence, liquid biopsy is introduced with great promise for personalised medicine because of its ability to provide multiple non-invasive snapshots of the primary and metastatic tumours.

Keywords: Cancer, cell-free DNA, cell-free RNA, circulating tumour cells, liquid biopsy

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Submitted: 04-Jun-2022, **Revised:** 16-Oct-2022, **Accepted:** 16-Dec-2022, **Published:** 21-Mar-2023

INTRODUCTION

Tissue biopsy is always a "gold standard" for diagnosis and treatment choice in cancer patients. Biopsy is a procedure for obtaining tissue samples from a lesion for detecting a pathological process. Traditionally, a biopsy could be classified as needle biopsy, punch biopsy, and incisional and excisional biopsy. Taking a tissue biopsy is thus generally an invasive procedure.[1] Hence, repeated biopsy at various stages throughout the clinical course of any carcinomatous disease is quite impossible.

Also, there are certain limitations that render tissue biopsy an unfeasible option for long-term monitoring, lacking information regarding spatial and temporal heterogeneity of the tumour. Moreover, tissue biopsy cannot be performed when clinical conditions have worsened or because of limited accessibility of tumour tissue. It also

Access this article online

Quick Response Code:

Website: www.jomfp.in

DOI: 10.4103/jomfp.jomfp_251_22

How to cite this article: Tomar U, Grover N, Tomar S, Bhalla K, Singh S. Liquid biopsy and its significance in tumour – Detection in the field of pathology. J Oral Maxillofac Pathol 2023;27:195-200.

demands high total costs because of the complexity of the medical procedure, needing dedicated personnel, a medical surgery room available, and a longer recovery time.[2]

As molecular biology is improved nowadays, the treatment of cancer has remarkably improved simultaneously because of increased knowledge of the molecular abnormalities that drive human cancer growth. This gives rise to the development of ever more effective targeted cancer therapies. In emergence of these advancements, the testing of molecular biomarkers for cancer patients has become mandatory for successful treatment.[3]

Hence, the concept of liquid biopsy is introduced to oncopathology, which has the capability to revolutionise the successful management of onco-patients, eliminating the need of invasive procedures to obtain tissue samples for biopsy and provide information on therapy response.[4] Liquid biopsy has been recently drawing attention among pathologists as an adjuvant and complementary tool to tissue biopsy as it is a minimally invasive diagnostic tool capable of assessing the genetic landscape of solid tumours.[5]

WHAT IS LIQUID BIOPSY

It is a new concept in the diagnosis, management, and predictive behaviour of a tumour with a non-invasive technique.[4] Liquid biopsy was originally described by National Cancer Institute as "the test on the blood sample of the patient to study the circulating tumour cells or cell-free deoxyribonucleic acid (cfDNA) derived from the tumour cells in the blood".[5] In recent years, liquid biopsy has played a significant role for real-time monitoring of the various aspects of the tumour during its clinical course. It is one of the most emerging and promising technologies in the field of oncopathology.[5]

In this review, we discuss the various aspects of liquid biopsy including its components, detection, analysis, clinical applications, and future prospects.

COMPONENTS OF LIQUID BIOPSY

Any parts of the tumour shed materials into the body fluids, be their nucleic acid, protein, secretory vesicles (such as exosomes), or tumour cells. Mostly during the initial period of oncogenesis and metastasis, the primary tumours shed the whole cell, cfDNA, or exosomes [containing messenger ribonucleic acid (mRNA), micro-RNA (miRNA) or small RNA sRNA], which carry vital information of the primary tumour.[5] With more advanced stages of cancer, it sheds more cancer cells. Therefore, the more likely tumour cells and cancer-causing DNA can be found in the blood. Liquid biopsy techniques detect these different blood-based biomarkers including circulating tumour cells (CTCs), circulating tumour DNA (ctDNA), also known as cfDNA, circulating RNA (cfRNA), and exosomes.[6]

Thus, a liquid biopsy may provide an even more, representative sampling of the biomaterials from cancer. The body fluids from which we can detect these biomarkers include not only peripheral blood but also other fluids, such as urine, cerebrospinal fluid, or effusion fluids. However, it is most commonly referred to peripheral blood, and the current review is focused and limited to this scope.[1]

DEFINITION

Liquid biopsy is the real-time analysis of tumour cells or tumour cell products [e.g., cell-free circulating nucleic acids (ct DNA, cf RNA), extracellular vesicles or proteins], which are shed into the blood or other body fluids by primary or metastatic tumour lesions. Isolation of these tumour-derived components from peripheral blood and other body fluids and their genomic or proteomic assessment represent a new diagnostic tool in the world of oncopathology, which has been called 'liquid biopsy', Figure 1.[5]

METHODOLOGY

Obtaining the sample

Liquid biopsy is obtained by a routine blood sample and requires only a small amount of blood, that is, 6–10 ml. It takes less time, and its cost-efficiency for sample taking helps in gaining knowledge about the spatial and temporal heterogeneity of cancer. It also allows real-time monitoring during the clinical course of treatment. These circulating molecules can be detected in various biological fluids including main blood but also in urine, saliva, cerebrospinal fluid, and seminal plasma, [Figure 2].[7]

A detailed description of every component is as follows:

Circulating tumour cells

American Society of Clinical Oncology (ASCO) has recommended CTC as a biomarker. CTCs are released from solid tumours into the circulation of cancer patients at any stage of the disease or by distant metastatic lesion, so they obtain most of the mutational profile. They can circulate individually or in clusters. In order to get into circulation and form metastases, they must undergo a multistep process, called "metastatic – cascade". These

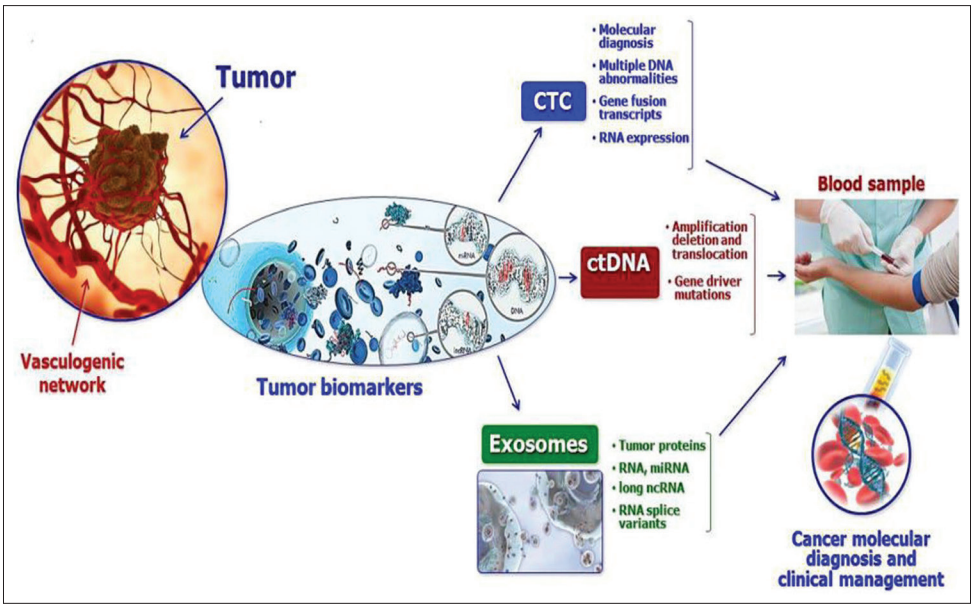

Figure 1: Molecular cells (CTCs), circulating tumour applications of ctDNA, and exosomes as a liquid biopsy for personalised medicine.[5]

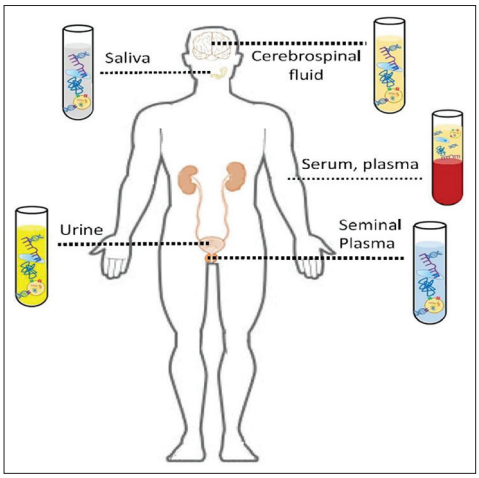

Figure 2: Circulating molecules in various body fluids[7]

cells can be isolated by means of density gradient centrifugation, size centrifugation, fluorescence-assisted cell sorting, or antibody-conjugated microfluidic devices [Table 1].[1]

Circulating cell-free DNA and Tumour DNA

The circulating cfDNA in humans was first reported in 1948 by Mandel and Metais. Two main sources of origins of cfDNA are (i) the cellular breakdown mechanisms and (ii) the active DNA release mechanisms.[2] The concentration of the total cfDNA in healthy individuals is on average 30 ng/mL plasma and ranges from 0 to 100 ng/mL, whereas in cancer patients, this can be up to 1,000 ng/mL with an average of 180 ng/mL[2] ctDNA isolated in blood and all other body fluids. There are three main potential sources of ctDNA: (i) apoptotic or necrotic tumour cells, (ii) live tumour cells in tumour tissue, and (iii) CTCs 6. It is estimated that patients with a tumour load of 100 g in size release 3.3% of the tumour DNA into the circulation on a daily basis[2] and that the half-life time of ctDNA in the blood circulation ranges from 16 min to 2.5 h. The rate of ctDNA shedding into the circulation depends on the location, tumour burden, size, and vascularity of the tumour, cancer stage, cellular turnover, and response to therapy [Table 2].

Table 1: Advantages and Disadvantages of circulating tumour cells

Advantage	Disadvantage
1. Phenotypic and genotypic analysis	Only 1–10 CTC/10 ml of blood,so derivation of result could be practically much difficult
2. Count significantly, correlates with prognosis	Fragility of cells made retrieve the circulating tumor cell difficult
3. High specificity	False negative and positive results
4. In vitro culturing and analysis	

cfDNA can be removed from the blood by simple phenol/chloroform extraction technique, and detection was performed by using various dyes, such as SYBER Green I, and polymerase chain reaction (PCR) assays. The fluorometric assay provides an estimation of the total cf DNA content, whereas PCR-based assay helps in the detection.

Exosomes

Exosomes are membrane-bound vesicles of 40–150 nm in diameter. These are of endosomal origin as they are produced within the endoplasmic networks and secreted by cells, working as mediators of intercellular communication. They are secreted from both the normal and tumour cells and are found in both blood and in the various body fluids.

Table 2: Advantages and Disadvantages of cfDNA and ctDNA

Advantages	Disadvantages
1. High sensitivity 2. Allows DNA sequencing and methylation 3. Can decrease diagnosis bias from tumour heterogeneity 4. Can timely and dynamically monitor tumour progress	1. ctDNA is unstable and requires fast processing 2. Low specificity because of cfDNA 3. False positive/negative results

They are most abundant in the blood, and they bear the surface markers of the original cells. The exosomes can be extracted with the help of antibody-based arrays, immune affinity-based capture technique, and microfluidic chip methods.[2] These contain several types of parent cell-driven bioactive molecules, such as proteins, lipids, mRNAs, miRNAs, long non-coding RNAs, and genomic DNA. These are thriving research hotspots in the field of cancer as they carry much tumour-specific valuable information.[8]

ANALYSIS OF LIQUID BIOPSY

- EXTRACTION OF MOLECULAR INFORMATION

The first and foremost step in attaining valuable information pertaining to cancer treatment is efficient isolation of CTC, cfDNA, and cfRNA shed by the tumour cells, which are very few in amount, in order to gain information and increase sensitivity and specificity.[6] Molecular analysis should be performed, followed by isolation; it includes fluorescence *in situ* hybridisation (FISH), microarray, sequencing, immunofluorescence, flow cytometry, and reverse-transcriptase PCR (RT-PCR). Digital PCR comprising droplet-based systems (ddPCR), microfluidic platforms for parallel PCR, NGS, BEAMing (Beads, Emulsions Amplification and Magnetics), and amplification-refractory mutation system (Scorpion-ARMS) assay are additional techniques that can also be considered.[6]

- EPIGENETIC STUDY

DNA hypermethylation in the promoter region can be assessed by methylation-specific PCR protocol. Microarray-based DNA methylation assay technology is very fast and helps in the extensive assessment of DNA methylation in cfDNA.[8]

CLINICAL APPLICATIONS OF LIQUID BIOPSY

Capture of CTCs, ctDNA, and exosomes as a "liquid biopsy" has several promising advantages over standard biopsy pertinent to clinical settings [Figure 3].[6,8]

- Early Diagnosis and Screening of Cancer

A simple quantitative estimation of cfDNA gave information to assess the presence of cancer. Several studies have highlighted the presence of a higher amount of cfDNA in the blood of cancer patients as compared to the healthy control.[8]

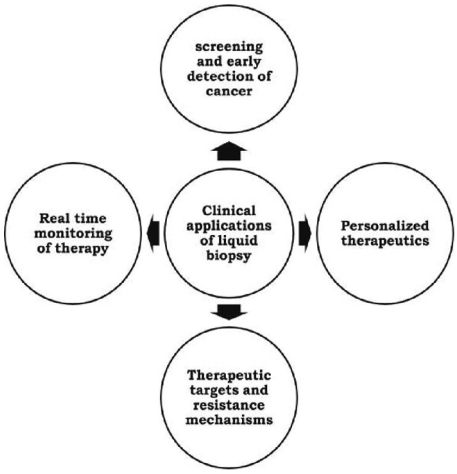

Figure 3: Clinical application of liquid biopsy for personalized medicine[6]

- Detection of Tumour-Specific Mutation

Various tumour-specific DNA mutations may be detected in the liquid biopsy sample to express the probability of cancer.[8]

- Tumour-Specific miRNA and sRNA:

miRNA and sRNA can be analysed; they detect the early carcinomatous representation. Therefore, these biomarkers are also important in the diagnosis and detection of any cancer.[8]

- Monitoring Minimal Residual Disease:

A liquid biopsy provides the presence of minimal residual diseases in various carcinomas in post-surgical patients. They also suggested that the detection of these mutations, which are expressed by ddPCR, may be helpful to detect minimal residual disease after treatment.[8]

- Emerging Analytes for Liquid Biopsies[9]

The behaviour of platelets has been modified because of tumourogenesis, and this behaviour alteration is called as tumour-mediated platelet education. Biomarkers which are associated with these altered platelets are an emerging way of the non-invasive method of diagnosis of any tumour. Analysis of RNA-Seq of these educated platelets is a promising tool which diagnoses early and advanced stages of cancer.[9]

ADVANTAGE OF LIQUID BIOPSY

It is a non-invasive test with minimum complication which serves as a method of overcoming intra-tumour

heterogeneity. Analysis with ctDNA and CTC is not susceptible to size-dependent visual resolution, limited needle access, user-dependent error, potential radiation exposure, invasive complications (haemorrhage, infection, tumour seeding), and tissue-sampling error. A repetitive sample is possible in liquid biopsy. Many patients with limited access to biopsy would be able to undergo necessary diagnostic testing. At the advanced stage of cancer, treatment and survival information could not be collected, but mutations that can be targeted with treatment were reported and discussed.Therefore, the role of liquid biopsy would be helpful in these conditions.[10]

LIMITATIONS OF LIQUID BIOPSY

Although liquid biopsy is an advanced technique for tumour detection, it also has a few drawbacks. The main disadvantage is the absence of standardisation of the methodologies and an often insufficient technical and clinical validation for accurate clinical implementation.[2] For example, most studies have been conducted only on small cohorts of patients, and large-scale investigations are needed to confirm liquid biopsy's capabilities, particularly in the reference of CTCs. A broader way of approaches has been utilized for enrichment and detection of liquid biopsy biomarkers which subsequently increase the variance of results, but standardisation of methods is essential.[9] The accuracy of these diagnostic tests is majorly influenced by the quality as well as quantity of the DNA extracted from the body fluids. There is the possibility of missing the mutation in the case of contamination or widespread necrosis or when minute quantities of DNA are available.[6]

Another limitation of liquid biopsy is the presence of mutations at higher frequency in cfDNA in benign or premalignant conditions compared to their malignant and higher-stage counterparts.[6] Blood processing delays, storage, temperature, agitation of the sample, and shipment are also a major source of variability among samples. Also, the choice of anti-coagulant used in plasma collection can influence downstream detection technologies, such as quantitative RT-PCR [Table 3].

Table 3: Comparative analysis of liquid biopsy vs conventional biopsy[6,8]

Factors	Liquid Biopsy	Conventional biopsy
Cost of sample collection	Cheaper	More costly
Sample preparation	Difficult and needs a sophisticated instrument	Relatively easy
Real-time monitoring	Possible	Not feasible
Minimal residual disease detection	Possible	Not feasible
Personalised treatment	Possible	Possible
Archival tissue preservation	Not always possible	Possible But not in FNAC
Study of tumour heterogeneity	Possible	FNAB may be performed from multiple sites but not in conventional biopsy
Clinical sample	Blood and other body fluids	Effected tissue
Risk	Minimal	Depends on location of tumour
Invasive	Minimal Invasive	Invasive
Time for recovery of patient	Quick	Time-intensive

CONCLUSION

In the era of precision medicine, successful treatment is based on tumour behavior rather than its average response to management.[7] Molecular profiling, the analysis of genomic, transcriptomic, and/or proteomic profiles, represents a critical pre-requisite for the successful development of treatment strategies. Liquid biopsy is a promising non-invasive method for molecular profiling, enabling assessment of cfDNA and other circulating molecules in various biological fluids for biomarkers. Although technically challenging, an advantage of liquid biopsies over other traditional tissue-based biopsies is the enablement of longitudinal monitoring which could help clinical oncologists gain a broader molecular understanding of the disease.[6]

So far, the most exciting applications of liquid biopsies seem to be prognosis and early assessment of treatment failure in successful management of carcinomas.

Financial support and sponsorship
Nil.

Conflicts of interest
There are no conflicts of interest.

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