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LIQUID BIOPSY- A COMPLEMENTARY APPROACH TO TISSUE BIOPSY

Investigation Techniques

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ABSTRACT : Carcinoma is a complex, heterogenous and dynamic disease involving multiple gene interactions that affects numerous biological pathways. Liquid biopsy is a simple and non invasive alternative to surgical biopsies involving sampling and analysis of non solid biological tissues primarily blood. Through interrogation of tumor derived materials namely circulating tumor cells(CTCs) and circulating tumor DNA(ctDNA), it enables doctors and clinicians to discover the range of information about the tumors and thus choose the right treatment for the patient at the right time in addition to monitoring the relapse chances also. Procedure is quicker than traditional biopsy. In this review paper we present an overview of the advantages, challenges, clinical applications and contribution of liquid biopsy in early identification, prognosis and treatment response of patients to various neoplasms.

Keywords:
Circulating tumor cells, Circulating tumor DNA, Liquid biopsy, Cancer diagnosis

Conflict of interest: Nil
No conflicts of interest : Nil

INTRODUCTION : Cancer detection by means other than X-rays, CT scans, MRI, colonoscopy, mammography and PAP smears has been a subject of research in the past recent years[1]. Biopsy is a sample of tissues or cells taken from almost any part of the body and sent to the lab to check for carcinoma. Although surgical biopsy is a gold standard for diagnosis and treatment choice but it has it's limitations in terms of [2].

1) Tissue is the issue- i.e. insufficient tissue material for genomic testing(tissue inadequacy)
2) Patient intolerability and inconvenience.
3) Local trauma to tissues (invasive).
4) Sampling of only a part of tumor.
5) Captures a fraction of heterogenicity.
6) Don't show all mutations.

To overcome these shortcomings, the field of cancer and its related treatment amenities are researched and influenced by latest developments, one such being liquid biopsy[1].

US National Cancer Institute (NCI) defines liquid biopsy "as a test done on a sample of blood to look for cancer cells from a tumor that are circulating in the blood or for pieces of DNA from tumor cells that are in the blood"[3]. It is a non tissue based diagnosis that acts as a minimal invasive alternative to tissue biopsy and involves sampling of non solid biological tissues (fluid or liquid) so also called as fluid biopsy. Liquid in this case is blood[1].

REVIEW OF LITERATURE

(A) SAMPLES

1) Blood- either whole blood or cell free plasma is used as a sample. Sample volume required is only 5ml which is then spun down to get 2ml of plasma to analyse blood markers. Kits or EDTA tubes are used for sample collection[4].

2) Urine- small fragments of DNA gets accumulated in the urine where the mutations of interest are identified and quantified. The advantages of urine sample are

-convenience as patient can self collect the sample at home or clinic without the help of
medical professional
-No refrigeration is required
-less infection risk
-Frequency of testing as unlimited volume is available
-Completely non invasive[4]

3) Other body fluids- like CSF, saliva, amniotic fluid, fluid

from respiratory tract, fluid from gastrointestinal tract, etc[4].

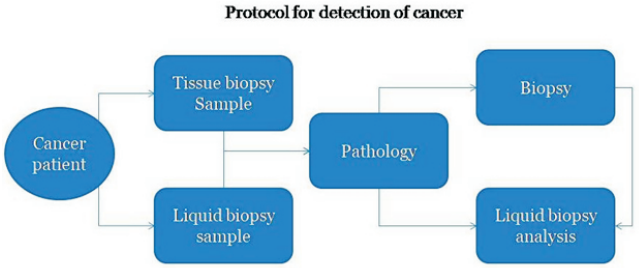

Figure 1- Protocol for detection of cancer

(B) LIQUID BIOPSY BIOMARKERS

After the collection of sample, next phase is the identification and analysis of various biomarkers for conformation and detection of cancers (figure 1). The important biomarkers are3-

1) Circulating tumor cells (CTC).
2) Circulaating tumor DNA (ctDNA).
3) Other tumor derived markers like exosomes and tumor educated platelets.

CTC-

These are the cancer cells that get detached from primary tumor or metastatic lesions and get lodged at other sites in the bloodstream. Mainly used to assess the likelihood of patient response to given therapy before the treatment is administered. 0 to 10 CTC/ml of blood as single or clusters of cells is considered diagnostic5 (figure 2). Techniques for its detection are6, 7-

- Antibody capture
- Size exclusion
- Depletion of red and white blood cells
- Dielectrophoresis
- Immunostaining
- Fluorescence in situ hybridization
- RNA sequencing

ctDNA-

It is a more common biomarker .Cells in the body die continuously and are replaced by new cells but cancer cells die at an increased rate. ctDNA is derived from tumor cells undergoing apoptosis or necrosis (figure 2). It represents a fraction of total cell free DNA circulating in patient blood. Cancer patients have much high level of ctDNA than healthy individual. ctDNA is <0.1-10% of total cell free DNA in human blood8. When tumor cells increase in volume so number of apoptotic and necrotic cells also increases leading to release of ctDNA in bloodstream mixed with normal circulating cell free DNA. Mainly useful in detection of breast, lung, ovarian and prostate carcinomas but is expected to have an impact on all types of carcinomas. In non small cell lung carcinoma(NSCLC), due to insufficient material for surgical biopsy it has an important role to detect T 790m resistance mutations in epidermal growth factor receptor gene for targeted treatment[9]. Techniques for detection of ctDNA are6

- quantitative PCR amplification
- Digital PCR
- Droplet PCR
- Targeted deep sequencing
- Next generation sequencing

EXOSOMES : These are small sized extracellular vesicles of endocytic origin that plays a key role in cell to cell communication both in physiologic and pathologic processes and are released in different body fluids. Exosomes contains proteins, DNA and RNA from patient tumor10 (figure 2)

TUMOR EDUCATED PLATELETS:

Platelets are anucleated formed elements of the blood who derive their mRNA from megakaryocytes during platelet origination and are involved in hemostais and wound healing initiation[10,11]. Tumors educate platelets by altering their mRNA profile. When platelets confront cancer cells they release tumor associated molecules (education) and splicing signals which causes sequestration of circulating mRNA and provides a scan for molecular traces of DNA[12].

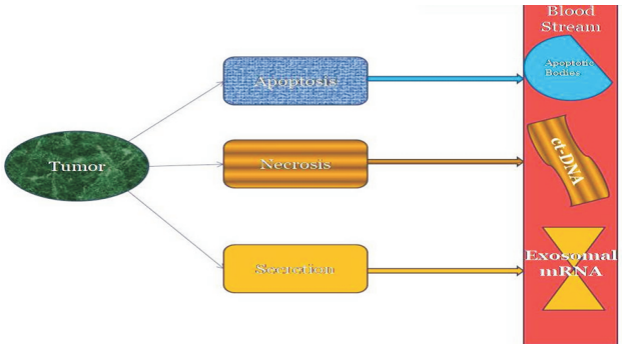

Figure 2 – various biomarkers used in liquid biopsy

APPLICATIONS (figure 3)

1) Diagnostic tool for early tumor detection even before the sign and symptoms appear (screening).
2) To access the likelihood of patient to respond to given therapy before the treatment is administered.

3) Monitoring of patient for advanced carcinoma after they receive treatment.
4) To know about the kind of molecular changes taking place in a tumor during the course of treatment.
5) Monitoring tool for relapse and reoccurrence.
6) Monitoring of genetic changes and mutations.

Detection of resistant mutations and resistance to treatment[9].

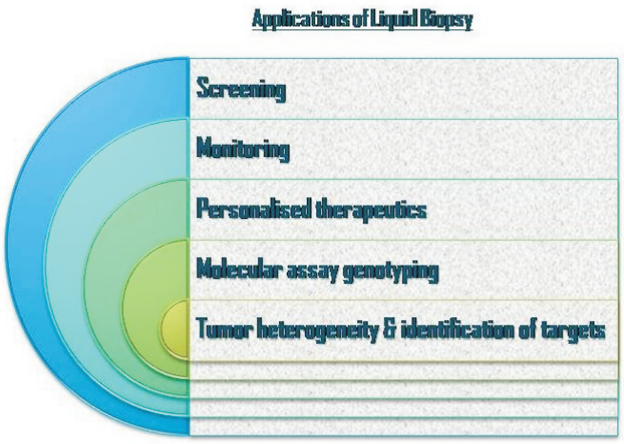

Figure 3- Applications of liquid biopsy

LIMITATIONS

1) Less specificity and sensitivity.
2) No detection of heterogeneity.
3) Pregnant women have DNA from their baby placenta in blood and patients of heart attack and stroke also contains DNA fragments (circulating endothelial cells) so differentiation with other types of DNA is must to avoid false alarm as very small amount of ctDNA is present in bloodstream[3].

DISCUSSION AND CONCLUSION : Although solid biopsies will continue to have a main role in cancer management but the use of tissue specimens is limited as they have an invasive protocol whereas ctDNA in blood detects tumor genetic alterations in a non invasive manner thus liquid biopsy has the potential to revolutionarize cancer care and is considered as the dawn of a new era of cancer therapeutics. It is complementing rather than alternative for extracting valuable information about genetic features of any individual tumors thereby offering varied range for early detection, prognosis and monitoring the treatment response and resistance. Being non invasive addition to surgical biopsy, the provision for serial sampling gives an overview about heterogeneity and plays a vital role in predicting the cancer reoccurrence earlier than conventional techniques. Information retrieved and gathered from various biomarkers of liquid biopsy can be linked and integrated with each other and also with other protein markers so as to obtain better and improved results. In this way, liquid biopsy acts a companion diagnostic for molecular monitoring and staging.

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