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Liquid Biopsy: *A New Era in Lung Cancer Management*

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Abstract:

Cancer is associated with multiple genetic mutations and analysis of these is increasingly used for management. Mainly histopathological examination of biopsy specimen is used for decision but sometimes biopsy specimen cannot be available due to invasive nature of surgical procedures and inaccessible tumor site. However tumors are highly heterogeneous and evolve over time and can alter their molecular genotype making clinical decisions based on historical biopsy data suboptimal. Tumor cells release circulating free DNA (cfDNA) in blood. This can help to detect genetic aberration. A liquid biopsy can provide information's from blood samples or body fluids and it is non-invasive. It can help to screen, diagnose and treatment in a personalized way and also detect mutations over time.

Key words: *Cancer, DNA, liquid biopsy.*

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Introduction:

Biopsies have been used by clinicians to diagnose and manage disease for 1000 years[1].

The science of non invasive disease monitoring has advanced greatly since circulating cell free DNA (cfDNA) was first reported in body fluids by mantle and Metais[2].

Since then, the evolution of sensitive cfDNA detection technologies has enabled the development of liquid especially in oncology, Liquid biopsy allows for patient stratification, screening, monitoring treatment response and detection of residual disease or recurrence.

Malignant cells are heterogeneous in behavior. Liquid biopsies have grown in importance become the genetic profile of tumors can affect how they will respond to a certain treatment. There is also limitation of detection of single snap shot of the tumor-. This single biopsy bias was highlighted in a study by Gerlinger et al[3].

It was found that there is marked intratumoral and intertumoral evolution when biopsies where taken from different parts of a primary tumors and its metastases. But there are many difficulties in obtaining a tissue biopsies as many as 3% of cases

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do not have accessible tissue[4].The hazards are discomfort of the patient, clinical risk to the patient, potential surgical complications and economic consideration. All the tumors may not be accessible for biopsy. Even biopsy procedure may cause seeding cancer to other sites[5].

In 1948, circulating free DNA (cfDNA) detection was the first step toward liquid biopsy[2]. It was also found that cfDNA levels were higher in patients than the healthy individuals. So it can be used for screening the disease through a simple blood test[6]. The heterogeneous landscape of the tumor was also possible to be detected by using a blood sample[7].

Where a tissue sample was taken for biopsy it only informed the genotype at that particular point but it is known that tumors are very dynamic and change their dominant mutation pattern or may acquire new mutations. This is particularly unfavorable when stratifying patients to a specific targeted therapy.

Approximately 50% of NSCLC patients become resistant to tyrosine kinase inhibitor therapy though an epidermal growth factor receptor (EGFR) T790M mutations[8,9] whereas only <5% of NSCLC patients have this mutations detectable in the primary biopsy[10]. So liquid biopsy helps in early diagnosis of cancer by cfDNA assessment[11,12] and also tumor-associated genetic alterations can be detected that will help in the assessment of prognosis, early detection of disease recurrence and predicting response to particular treatment.

How to perform the liquid biopsy

Presently the most commonly used protocols to obtain cfDNA requires approximately 1 ml of serum or plasma (3 ml of blood) and preparation should not exceed 4-5 hours following the blood draw. For plasma preparation, blood must be collected in a tube treated with an anticoagulant preferably EDTA (ethylene diamine tetra acetic acid). Cells are then removed by centrifugation and the supernatant or plasma is removed[13]. Serum is collected after the blood is allowed to clot and following centrifugation is the supernatant or serum is removed[14]. Circulating DNA is then extracted from the plasma or serum using commercially available kits.

A portion cfDNA is derived directly from the tumor (ctDNA) also from circulating tumors cells (CTC) and this fraction can be quantified[15,16].

Furthermore, and in vivo study has shown a direct correlation between tumor burden and the quantity of ct DNA released.[17] The fraction of circulating DNA that is derived from the tumors can range between 0.01% and 93%[18,19].

Techniques are available for reliable monitoring of tumour-associated genetic aberrations including somatic mutations, loss of heterozygosity and chromosomal aberrations in the blood[20].

Use of liquid biopsy

Assessment of prognosis:

Assessing prognosis for an individual patient involves a combination of clinical observations staging, and histopathology of different tumor types. In this context liquid biopsy may be useful in circumstances where a tissue biopsy in not available or genetic analysis of archived tumor samples is not possible[21].

Studies have shown that there is a statistically significant correlation between disease stage and the presence of tumor associated genetic aberrations in the blood of patients with different cancers like breast, pancreatic, colorectal and oral squamous cell carcinoma[22].

The presence of tumor-associated genetic aberrations including tp53 mutations and loss of heterogygosity, correlated with overall survival or disease-free survival.

Liquid biopsy is much more useful in patient with unrespectable advanced stage disease. In a multi variant analysis, KRAS mutations present in the plasma of 246 patients with advance stage NSCLC was shown to predict poor prognosis in patient receiving first line chemotherapy[23].

Detection of recurrence:

A promising clinical implicating of liquid biopsy is the early detection of relapse after potentially

curative treatment. After treatment with curative intention, patients are monitored for signs of residual disease and local or distant recurrence using radiological image during post treatment follow-up[24-26].

But the disadvantage of these techniques is cost, requiring contrast media (exposing patient to doses of radiation) and control be used for frequent monitoring. It also has limited sensitivity for the detection of micrometastases[27,28].

In a land mark paper Diehl et al. showed that by monitoring tumor specific aberrations (including APC, TP. 53, KRAS) is the plasma of patients it was possible to identity disease recurrence with almost 100% sensitivity and specificity. Patients with residual diseases were also identified based on the. Persistence of tumor associated genetic aberration in cfDNA immediately after surgery.

Difficult-to-diagnose cancers:

Liquid biopsies can be used to assist in the clinical management of difficult-to-diagnose patients with advanced stage cancer, as in the case of bone metastases. For example, in a study in which targeted deep sequencing of cancer-related genes (including TP53, PIK3CA and KRAS) was carried out on cfDNA in a patient who had previously undergone surgery to resect synchronous cancers of the bowel and ovary, it was shown that on relapse the metastases was derived from the original ovarian cancer (owing to the presence of a R273H TP53 mutation). A biopsy in this case was not possible and had the information derived from cfDNA been available immediately unnecessary delay or uncertainty over treatments might have been avoided.

Prediction of response to treatment:

The presence or absence of a single genetic alteration in tumor DNA is currently employed to guide clinical decision making for a number of targeted agents, for example EGFR mutations for gefitinib in NSCLC; ALK rearrangements for crizotinib in NSCLC[29]. Targeted agents are often used or tested in patients with advanced-stage disease having multiple metastases. Sometimes a new biopsy cannot be obtained and ctDNA might provide superior molecular information compared to archival tissue DNA for determining the current cancer molecular status. A liquid biopsy could obviate the need for tumor tissue DNA in metastatic patients.

Conclusion:

Cancer is a complex and dynamic disease that can change quickly. To fully deliver on the promise of personalized medicine, development of reliable and robust non-invasive platforms for the diagnosis, patient stratification and to monitor treatment response are paramount. Although liquid biopsies have a great potential, many hurdles must be overcome before proceeding to the clinic. The various liquid biopsy platforms described in this review have the potential to add tremendous value to the care of cancer patients.

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