Review Article DOI: 10.18203/issn.2456-3994.IntJMolImmunoOncol20172637

Liquid biopsy, an innovation of modern medicine

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ABSTRACT

Biopsies have been the best tool in the hands of the clinicians for the appropriate management of malignancies including diagnosis, prognosis and reassessment. They are and have been the gold standard in their diagnosis. Due to certain unavoidable limitations of needle biopsy, there is emergence of a new biomarker in oncology called LIQUID BIOPSY: Analysis of circulating tumour cells (CTC) and circulating free tumor DNA (ctDNA) from the peripheral blood. As this technology evolves it may prove to be a critical component of personalized medicine in this modern era of diagnostics. Liquid biopsy has a great potential in cancer diagnosis, monitoring and predicting survival. Nevertheless false positive results exist. The major challenge with this technology is assay sensitivity and specificity. This mini-review tries to fill the gap between reality and our hope of this "liquid biopsy".

Key words: Biopsy, Circulating tumor cells, cfDNA

Introduction

Biopsies have been the best tool in the hands of the clinicians for the appropriate management of malignancies including diagnosis, prognosis, and reassessment. They are and have been the gold standard in their diagnosis. Due to certain unavoidable limitations of needle biopsy [Table 1], there is emergence of a new biomarker in oncology called LIQUID BIOPSY: Analysis of circulating tumor cells (CTC) and ctDNA from the peripheral blood. As this technology evolves, it may prove to be a critical component of personalized medicine in this modern era of diagnostics.

The tumor tissue is usually not an issue for early, resectable, and approachable cancers. However, there are some inherent deficiencies with biopsies in inaccessible or advanced malignancies. Modern personalized cancer medicine is largely dependent on finding molecular targets. Intratumoral heterogeneity is an enemy of single biopsies as was scientifically illustrated by Gerlinger *et al*.[1] when they showed that gene expression signatures differed significantly between biopsies taken from different sites of the primary tumor as well as those from different metastases. Multiple biopsies take intra- and intertumor heterogeneity into consideration but may be difficult, risky, painful, and can potentially cause tumor seeding[2] and fistulas.

Liquid biopsy has a great potential in cancer diagnosis, monitoring, and predicting survival. Nevertheless, false positive results exist. The major challenge with this technology is assay sensitivity and specificity. Several hurdles still exist such as the lack of consensus in technical approaches of choice, preferable sample type, storage conditions, candidate molecules, and suitable detection technique [Table 2]. This mini-review tries to fill the gap between reality and our hope of this "liquid biopsy."

What is Liquid Biopsy

Small amounts of circulating free DNA (cfDNA) has long been known to be present in blood and more so in disease than in healthy individuals.[3,4] The tumor DNA enters circulation from apoptosis and necrosis of tumor cells at the site of origin with a small contribution from the dying CTCs. Likewise, ctDNA is also present in blood which can be differentiated from non-tumor cfDNA by their mutation profile and specific somatic mutations.[5] The proportion of ctDNA to the total blood free DNA differs according to the location of cancer (low in Central Nervous System tumors), stage of cancer (higher in advanced cancers), type of cancers (eg low in mucinous cancers), and detection methods (methods used and genetic aberration identifiers used). ctDNA is identified and quantified using appropriate targets such as KRAS mutation or PIK3CA mutation and various technologies [Table 3].

In addition to ctDNA, tumors also release CTCs into blood, which can be enriched using various technologies for detecting tumor cells (physical and biological properties such as size and expression of surface molecules, epithelial cell adhesion molecule, EPCAM).[6] The migration (and epithelial-mesenchymal transition, EMT) of CTCs from tissue of origin to blood and peripheral tissues is governed by the biological characteristics of the CTCs as well as the gradient of special chemokines, such as CXCR4 and CCR4. CTCs also have potentially same scope in cancer management as ctDNA. ctDNA has superseded the clinical advantage because of the sheer information that the ctDNA can provide through next generation sequencing in addition to the diagnosis, prognosis, and surveillance.

Table 1: Advantages of liquid biopsy

1. Ease of diagnosis: Liquid biopsies avoid invasive procedures, repeated biopsies and overcome the limitation of lacking enough tissue. It is helpful in inaccessible tumors and in patients who are unfit/or unwilling for invasive biopsy
2. Tumor heterogeneity: It gives more representative information of the tumor and has a potential to detect more targetable/actionable genetic aberrations
3. Prognostication: The amount of ctDNA and the mutational load as well as type of mutations fosters prognostication at the time of diagnosis
4. Prediction of response and effectiveness of anticancer therapy: The quantity of ctDNA, mutational load and specific mutations (appearance of new as well as reversion of old mutations) can be followed up prospectively which can help in therapy decisions
5. Reassessment: To detect residual disease or recurrence and or relapse
6. Monitoring the emergence of new alternative genetic pathways associated with resistance to current anticancer therapy as well as reversion of old active pathways which may warrant change of treatment
7. Difficult to diagnose cases

Table 2: Challenges of liquid biopsies

CTC and ctDNA

1. The tumor-associated genetic aberrations can be lost or gained over the monitoring period
2. The tumor-associated genetic aberrations can be lost or gained in response to treatment
3. The CTCs may detect aberration limited to clonal subpopulation
4. Identification of tumor DNA requires founder genetic aberrations (which are not lost during the progress of cancer) such as APC in colorectal cancer. These aberrations should not be affected by the anti-cancer therapy (passive mutations)
5. Standardization of the testing methods
6. Availability of standardized tests
7. Clinical trials of these tests with survival as endpoints

CTCs: Circulating tumor cells

Table 3: Techniques for the study of ctDNA[21]

Techniques used for the study of tumor genetic material (ctDNA)
Targeted sequencing:
Spectral karyotyping
FISH
Chromosome base CGH
Microarray-based CGH
SNP analysis
NGS
Targeted plasma re-sequencing (Tam-Seq)
MPS with PARE
Shotgun (MPS for identifying CNV and SNV)
WGS detects wider array of aberrations though with lower depth. It gives more detail regarding intronic/passenger mutations. It is at present prohibitively expensive for routine clinical use
WES is the analysis of exome (the part of genome which remains after getting transcribed and post-transcriptional changes and splicing

FISH: Fluorescent *in situ* hybridisation, CGH: Comparative genetic hybridisation, SNP: Single nucleotide polymorphism, NGS: Next generation sequencing, MPS: Massively paralleled sequencing, PARE: Personalised analysis of rearranged ends, CNV: Copy number variations, SNV: Single nucleotide variants, WGS: Whole genome sequencing, WES: Whole exome sequencing

The procedure for collecting the ctDNA/CTC sample is simple. About 1 ml of plasma or serum (3 ml of whole blood) is collected. They have a very short life in blood (few hours) and get cleared by spontaneous disintegration or extravasation into liver, bone marrow, and spleen.[7] Advances in the molecular tests such as targeted sequencing, *in-situ* hybridization, polymerase chain reaction (PCR), single nucleotide polymorphisms, targeted plasma resequencing (Tam-Seq), and whole genome/whole exome sequencing (WGS/WES) when applied to ctDNA can provide clinicians with a wealth of useful molecular information.

Clinical Application

Screening and early cancer detection

Current strategies for screening common cancers in high-risk individuals are grossly inadequate, and the role of newer technologies is being sought. The role of CTCs in the early detection of lung cancer in chronic airway disease patients was illustrated by Ilie *et al*.[8] Another study showed 50% of stage-I lung cancer patients have ctDNA.[9] Similar studies are going on in patients with inflammatory bowel disease. ctDNA can be detected in up to 73% of the patients with localized cancer, often without simultaneous CTC being present.[10] The role of CTCs and ctDNA detection is promising but still in need of significant improvements in specificity and sensitivity. The clinical application is far from reality at present except certain special circumstances.

Prognosis

The presence of CTCs in peripheral blood in patients with breast cancer was associated with poor disease-free survival, breast cancer-specific survival and overall survival with prognosis being worse in those with at least five CTCs in 30 ml of blood with cell search system.[11] The standardization of the cell search system led to the FDA approval of this system for the prognostication in metastatic breast, colorectal, and prostate cancer.[12]

CTC enumeration and characterization with certified systems provides reliable information on prognosis and may serve as liquid biopsy to identify therapeutic targets or mechanisms of resistance on metastatic cells such as mutations in KRAS in colon cancer or expression of the androgen receptor variant-7 in prostate cancer patients. PIK3CA mutation detected by digital PCR assay in breast cancer patients is an independent and significant prognostic factor.[13]

Detection of new therapeutic targets or reversion of old targets and primary and secondary resistance

Detection of resistance mechanisms is the heart of the precision medicine. Analysis of T790M by next generation sequencing of ctDNA in EGFR amplified NSCLC patient's resistance to 1st line TKI helps us to decide for further treatment in such

cases. Similarly, detection of mutations by liquid biopsy such as KRAS/NRAS in colon cancer, PIK3CA in NSCLC, BRAF in melanoma and resistant ALK in NSCLC are under clinical trials.

The importance of repeated Her2/Neu assessment in metastatic breast cancer cannot be over emphasized especially to detect conversion from negative to positive Her2 status. The detection of Her2 to this effect was successfully demonstrated by Gevensleben *et al*. using digital PCR.[14]

Real-time continuous assessment of treatment benefit

Prognostic value of the quantitative estimation of pre- and post-treatment CTCs in metastatic breast cancer was demonstrated in a pooled analysis of individual ctDNA. This also has a role in tracking clonal evolution and drug responses to targeted agents especially by following mutation such as KRAS, NRAS, BRAF, TP53, and PIK3CA.

Taking therapeutic decisions based on liquid biopsy

Clinical trials have an opportunity to prove the clinical benefit of taking therapeutic decisions based on liquid biopsies. Bidard *et al*. introduced the CTC measurements before and after first cycle of chemotherapy and randomized patients to stay on the same chemotherapy or switching to another therapy.[15] They confirmed the prognostic significance of CTCs in metastatic breast cancer patients. Another study (STIC CTC METABREAST) is ongoing to evaluate the role of CTCs in identifying hormone positive metastatic breast cancer patients who would benefit by early chemotherapy in comparison to hormonal therapy.[16] DETECT III study is exploring the role of anti-Her2 (lapatinib) in multiply treated metastatic TNBC patients with at least one Her^{2+} CTC/7.5 ml blood.[17]

The use of ctDNA has been successfully shown to predict the clinical benefit of erlotinib in patients with EGFR mutations detected on ctDNA leading to its FDA approval using the companion COBAS EGFR mutation test v2.[18,19]

Difficult to diagnose cancers

Inaccessible tumors such as deep pelvic, pancreatic, and bone tumors can be diagnosed with CTCs or ctDNA. There are case reports where synchronous versus metastatic malignancies have been identified by detecting different set of specific mutation.[20]

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How to cite this article: Gujree MA, Singh R. Liquid biopsy, an innovation of modern medicine. Int J Mol ImmunoOncol 2017;2:30-32.

Source of Support: Nil. **Conflict of Interest:** None declared.