PATHOLOGY AND LABORATORY MEDICINE
Openventio PUBLISHERS
Open Journal
http://dx.doi.org/10.17140/PLMOJ-1-102

Opinion

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Volume 1 : Issue 1
Article Ref. #: 1000PLMOJ1102

Article History
Received: January 26th, 2016
Accepted: February 1st, 2016
Published: February 1st, 2016

Citation
Chatterjee D. Liquid biopsies: handle with care. *Pathol Lab Med Open J.* 2016; 1(1): 3-6. doi: 10.17140/PLMOJ-1-102

Liquid Biopsies: Handle With Care

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Possibly the first mention of the term 'liquid biopsy' (as it is understood today) in the scientific literature appears in a report about the 7th International Symposium on Minimal Residual Cancer (ISMRC) held in Athens, Greece in 2009.[1] The symposium had focused on, among other things, Circulating Tumor Cells (CTCs) and their relationship to Cancer Stem Cells (CSCs). In that symposium, it was reported that Howard Scher of Memorial Sloan-Kettering Cancer Center described the application of CTCs in the evaluation of therapies directed at castration-resistant prostate cancer, as a 'liquid biopsy'. With time, the term has slowly gained popularity: as of 24th January, 2016, 237 PubMed articles have the term 'liquid biopsy' in the title or the abstract.

Despite the increasing number of publications, the exact definition of liquid biopsy is still in flux. When first used, it was used to refer to the diagnosis and characterization of solid tumors by harvesting and analyzing CTCs from blood. It was a reasonable (if fanciful) epithet, since it purported to look at the same tumor cells as a 'regular' solid biopsy, but extracted from a liquid sample. Post-harvesting, it also used some of the same techniques that regular biopsies used, like H&E staining, immunohistochemistry (IHC) and gene sequencing to detect mutations.

More recently, the meaning of the term liquid biopsy has been extended to the detection of tumor nuclear material in the blood. This has proved to be much more clinically attractive and generated unusually high interest in the scientific and business community. With this in mind, liquid biopsies can be defined as 'the analysis of blood and blood products to detect and analyze cells or nuclear material derived from a tumor'.

However, the same principle of non-native deoxyribonucleic acid (DNA) detection in blood can be applied for detection of fetal DNA in maternal blood for prenatal diagnosis of genetic anomalies in the fetus. Proponents of these assays have also used the term 'liquid biopsy'. Moreover, similar assays can also be conducted in other bodily fluids like Cerebrospinal fluid (CSF) and amniotic fluid. If these are brought into the fold, the definition broadens to 'the analysis of bodily fluids for the presence of cells and/or nuclear material for the detection of pathological conditions'. Note that the analysis of the same bodily fluids for a wide variety of other chemicals – proteins, pathogens, ions, gases etc. – do not fall under the current understanding of the term liquid biopsy.

Most of the current enthusiasm for liquid biopsies stem from the modern gene sequencing technologies that allow for the detection of cancer genetic material released to the circulation from dying cancer cells. Since some cancer cells can die right from tumor inception, in theory, liquid biopsies are capable of diagnosing the presence of cancer even when the tumor may be too small to be detected clinically or show up even on sophisticated imaging studies.

The genetic material is sometimes in the form of microRNAs (miRNAs), which are short single strands of non-coding Ribonucleic acid or non-coding RNA (ncRNA) that binds to target messenger RNA (mRNAs).[2] As early as 2002, it had been hypothesized that these translational and post-transcriptional regulators play an important role in cancer.[3] Other studies have shown that miRNAs can have prognostic value in a variety of cancers. It seemed only logical that cancer specific miRNAs can be used as biomarkers of cancer presence and type.[4]

http://dx.doi.org/10.17140/PLMOJ-1-102

Tumor DNA can also be used for cancer diagnostics. It has been demonstrated that analysis of circulating cell-free tumor DNA (ctDNA) can reveal the presence and type of cancer.[5] Proteins (like Prostate-specific antigen (PSA)) and RNA, although highly associated with cancers, can possibly arise from non-cancer cells. Circulating DNA carrying cancer specific mutations can only arise from cancer cells – this make it the most specific circulating biomarker for cancer in the body. This tumor DNA forms a small fraction of the total DNA in the blood (less than 0.1%). However, advances in speed, power and affordability of next generation sequencing has made it possible to sensitively detect ctDNA with high level of confidence. However, even with highly developed sequencing, ctDNA is not always detected in patient's blood. It is estimated that only 10% of patients with gliomas and 50% of patients with medulloblastomas and certain metastatic cancers have detectable ctDNA in the blood.

Circulating cancer cells are even less frequent than DNA (about 100 times less). Although this form of liquid biopsy was an interesting development in cancer diagnostics, it has been chronically plagued by two problems. The actual technology of mechanically harvesting cells did not work very efficiently, despite the use of a wide variety of cutting edge technologies. Secondly, by definition, when CTCs appear in the blood, the tumor has already progressed to the metastatic stage, especially if a good crop of CTCs is required for unambiguous diagnosis. This made the value of this detection rather less than satisfactory as a clinical tool.[6] Moreover, cells are more fragile and difficult to handle compared to DNA. This makes CTCs the least desirable of liquid biopsies, although a significant body of research has gone into investigating this option, and at least one CTC test has been approved by the Food and Drug Administration (FDA) (CellSearch) and is offered by Quest Diagnostics.

There is a distinct mismatch between the volume of scientific literature on liquid biopsies and the corporate enthusiasm for the new technology. In 2014, Guardant Health became the first company to commercialize a liquid biopsy test for cancer DNA in the United States. Their assay searches for 68 cancer genes in blood and is priced at about $5,400. Following Guardant's lead, an increasing number of venture capital backed startups have started offering DNA based liquid biopsy tests. Currently, the number of commercialized tests are about 10, with more in the pipeline. Interestingly, despite the price tag on these tests, as of last year, most insurers do not reimburse for liquid biopsies, as these are still considered investigational and unproven technologies. This has not prevented analysts at the venerable Wall Street firm JP Morgan from predicting that demand for liquid biopsies will rocket towards $20 billion annually in as short a time as 5 years, from about $100 million at the end of 2015.[7] That future honey pot has attracted significant amounts of investor dollars to startups working on liquid biopsies – Guardant raised $100 million for its test development, a figure similar to that raised by Grail, a spin-off of Illumina, the world's largest DNA sequencing company. Another startup, Pathway Genomics, having raised $130 million from investors, last year started offering its liquid biopsy tests to health individuals to check if they have insidious cancer, either as a single shot (at $699) or as quarterly scheduled tests (at $299, with subscription). The FDA has voiced serious concerns about the claims of the tests.[8]

The concept of a single blood test as a screening tool for a multitude of cancers is undoubtedly highly desirable. However, a sober, scientific and pragmatic outlook is also necessary in evaluating the potential of these tests in altering cancer management. This article touches upon some of the issues that will need to be resolved before liquid biopsies can truly be hailed as a revolutionary direction in cancer theranostics.

Cancer Screening

Liquid biopsies have been proposed as cancer screening tools in three clinical scenarios. First, they can be used as population screening tools of unmatched sensitivity, where tumors can be detected in the preclinical stage. Potentially, this will allow treatments of cancer to begin before they have reached the critical metastatic phase, dramatically increasing cure rates. Secondly, liquid biopsies can be used to test a patient's blood for cancer genes after treatment. Failure of disappearance of the genes after initial tumor removal would indicate continuing presence of the tumor either as involved margins or as micrometastases and guide further management. Third, the amount of tumor DNA in blood can be a prognostic factor for treatment outcomes. In a study of 30 women with metastatic breast cancer receiving systemic therapy, ctDNA levels showed a greater dynamic range, and greater correlation with changes in tumor burden, than either CA 15-3 or CTCs. It was also the earliest measure of treatment response in half the patients.[9] For those cases where treatment causes the DNA number to fall below detection limits, serial follow up monitoring can be a non-invasive method of catching recurrence early.

However, there are several caveats that have to be considered before adoption of liquid biopsy for cancer screening. Despite the universal adage that early detection leads to better cancer cures, it has not been clinically proven for a number of cancers. For example, despite the specificity of high PSA levels in the blood as a marker for prostate cancer, large clinical trials have demonstrated that earlier detection of prostate cancer through population screening of PSA does not actually have a statistically significant benefit to cancer survival. In many cases, the cancer is an indolent one, where the patient is more likely to die with the cancer than from it. At the other end of the scale, for highly aggressive cancers like pancreatic adenocarcinoma, absence of good treatment options mean that the prognosis is minimally affected by the time of diagnosis. It is as yet largely unknown which tumor mutation signatures are indolent and which are aggressive. The management pathway following a positive screen in an apparently healthy person is therefore complicated. Imagine a scenario where a liquid biopsy detects a genetic signature suggestive of a breast cancer. If clinical and radiologi-

cal tests can not pinpoint the lesion, it will raise a management dilemma: treat with high dose chemotherapy on the off-chance that there is a hidden tumor, or wait for the tumor to be clinically detectable?

There is thus a very real danger of over diagnosis and treatment. Many cancer biologists believe that the number of sub-clinical cancers is larger than the number of tumors that present clinically. These 'hidden' cancers are effectively kept in check by the body's immune surveillance system. If this proves to be the case, liquid biopsies might detect these brief tumorous conditions, leading to over diagnosis in the absence of clinical disease, and unnecessary treatment on the speculation of future tumor development.

On the other hand, given that not all tumors shed ctDNA at the same rate, what is the predictive value of a negative screen following initial treatment? Especially for tumors where ctDNA is detected in less than half the number of cases, would the absence of ctDNA be analogous to complete pathological response and trigger a reduction in vigilance?

Acceptance of liquid biopsies as cancer screening tools must be backed with clinical trials data clearly providing guidance in clinical scenarios.

Companion Diagnostics/Personalized Medicine

A second (and currently more common) use for liquid biopsies is to act as companion diagnostics – a specific diagnostic procedure (usually identification of a target molecule or gene in a tumor) that triggers treatment with a drug targeted towards that molecule. A common example is the detection of Her2 status of breast cancers prior to treatment with Herceptin. Liquid biopsies have been proposed to act as companion diagnostics by creating a genetic picture of the cancer from a few drops of blood. Genetic analyses of tumors are currently done from biopsy samples by molecular pathologists using next generation sequencing platforms. The rationale behind the tests is to identify specific genetic defects in the tumor to match the right drug to the tumor. There are about 50 anti-cancer drugs currently in the market that are targeted towards specific DNA defects, and personalized and targeted management of tumors may result in greater treatment efficacy. In 2012, it was claimed that genetic analysis of tumors resulted in specific therapeutic recommendations nearly 70% of the time.[10]

Moreover, tumors under treatment can sometimes change their genetic makeup when they recur. Instead of recurrent biopsies, successful use of liquid biopsies can help track these changes non-invasively, and change the treatment accordingly. Especially for tumors which are hard to biopsy, like lung cancer, liquid biopsies can significantly reduce morbidity from repeat biopsies. Even with the high price tag, the cost-benefit ratio of these tests are reasonable – since a course of cancer therapy can cost tens of thousands of dollars, a five thousand dollar test can save significant treatment expenditure if a drug can be ruled out as unsuitable prior to a therapeutic trial.

One of the earliest clinically approved companion diagnostic test using liquid biopsy was by the European Medicines Agency (EMA) for the detection of Epidermal Growth Factor Receptor (EGFR)-activating mutations in patients with non-small-cell lung cancer. Patients found to be positive for the mutation were considered for treatment with gefitinib, a drug with demonstrated specificity for the particular EGFR mutation. Prior to this, the EMA had approved the use of gefitinib in patients with a positive tissue diagnosis of EGFR, but later extended the indication to include liquid biopsies. A specific diagnostic assay was created by Qiagen which detects the specific mutation in circulating DNA.

However, even this use of liquid biopsies is not without controversy. Foundation Medicine (Cambridge, Mass), a leading provider of DNA tests on biopsied tissue samples, has gone on record stating that there appears to be a difference in the genetic signatures obtained from tissue and blood samples.[11] In the case of gefitinib for lung tumors mentioned above, the study that led to the regulatory approval noted that tissue biopsies tended to be more accurate than liquid biopsies. If there is indeed a significant chance of error between the tissue and liquid biopsy signatures, would the convenience of a non-invasive biopsy outweigh the risk of a wrong diagnosis?

The issue is further complicated when one considers that the genetic signatures obtained from different parts of a solid tumor may itself differ. Even with tissue based genetic analysis, tumors tend to yield a multitude of genetic errors. Just which parts of the tumor are shedding genetic material to the blood and does it matter? The answer is as yet unknown. Proponents of liquid biopsy claim that this procedure provides a much better aggregate picture of the whole tumor including metastases, unlike patchy physical sampling from tumor regions. However, this claim is yet to be proved.

Because of the challenge of tumor heterogeneity, the whole field of mutation guided personalized cancer management, for liquid as well as solid biopsies, is still under development. Molecular pathologists have proposed the concept of 'driver mutations' for specific cancers, which are proposed to be more important to tumor growth than other mutations. In theory, targeting the driver mutations will help keep the cancer in check. However, for many cancers, the specific driver mutations, if any, are unknown as of now. Both the American Society of Clinical Oncology and the National Cancer Institute have started clinical trials to test a slew of targeted chemotherapies to see if treatment guidance through DNA testing has a measurable effect on treatment outcomes. The results of these trials will help to better characterize the impact of companion diagnostics in cancer management.

CONCLUSION

Liquid biopsies are an exciting development in the management of cancer and other pathologies. It promises better clinical outcomes through earlier diagnosis and personalized treatments. However, much of these claims remain unproved in large scale double blinded clinical trials. The answers may be thrown up in the coming years as the new startups launch a series of clinical trials aimed at proving the value of their technologies. Pathologists and clinicians alike should keep these caveats in mind before adopting the new paradigm.

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