# Applying Liquid Biopsies to Cancer Biomarkers in Circulating Tumors

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**Lisa Tomcko:**

Welcome to the latest edition of the College of American Pathologists' CAPcast. I'm Lisa Tomcko, content specialist with the CAP. The liquid biopsy has rapidly emerged as a method to minimize the amount of tissue needed for testing, making it a less invasive and safer alternative to more traditional procedures. As it continues to evolve, liquid biopsies offer exciting new options in the practice of pathology. In this episode, I'm joined by Dr. Maria Arcila and Dr. JinJuan Yao pathologist at Memorial Sloan Kettering Cancer Center in New York City to discuss the development of this new diagnostic tool, Dr. Yao and Dr. Arcila, welcome to the podcast. Would you like to introduce yourselves?

**Dr. Maria Arcila:**

My name is Maria Arcila and I am a hematopathologist and molecular pathologist at Memorial Sloan Kettering. I do a lot of work in the laboratory and lately more specifically related to cell-free DNA and liquid biopsy. So this is a very, very timely topic.

**Dr. JinJuan Yao:**

Thank you, Lisa, for the introduction. I'm Dr. JinJuan. I'm a molecular pathologist of Memorial Lung Cancer Center.

**Lisa Tomcko:**

Great. Well, thank you both for coming on and to get straight into things. What are some of the advantages and disadvantages to using liquid biopsies?

**Dr. JinJuan Yao:**

Yeah, so liquid biopsy can test the different biomarkers in various disease settings. So for today's topic, we are going to focus on the testing of cancer biomarkers using molecular technologies to detect the genetic alterations of circulating tumor DNA, so-called ctDNA. The technology has both advantages and disa advantages. So one of the advantage, as you mentioned in the introduction, is non or minimally invasive, especially in comparison with tissue biopsy. It can be repeated multiple times during disease progress to capture tumor evolution, monitor treatment response, and identify drug resistance. It is a tool for cancer surveillance, not like tissue biopsy, which can only reprint the genetic alterations in that specific tissue. Liquid biopsy can capture intratumoral. Heterogeneity means that there's more than one clone of cancer cells within a given tumor, and the intra intratumoral heterogeneity the presence of different genetic alterations in different metastatic sites.

Liquid biopsy has advantage of high technical sensitivity when using next-gen sequencing with its unique clinical index in ultra deep coverage or using highly sensitive digital droplet PCR, the detection sensitivity can further be enhanced when using the tumor informed or semi tumor informed approach with the genetic alterations from tumor tissue available liquid biopsy can specifically search for those alterations and make a high confidence cost even if the fraction of ctDNA is very low. So liquid biopsy can be used as an alternative testing when tissue biopsy is not available, insufficient or feel the mutational profiling when tissue biopsy is not performing very well. So as for the disadvantages, I would say there's some limitations of the application of liquid biopsy because the liquid biopsy is not yet considered a standard testing procedure for cancer diagnosis. Tissue biopsy remains a gold standard for the confirmation and diagnosis of disease.

There's more of the supportive evidence for clinical utility is needed. There's currently not a widespread utilization of liquid biopsy testing within the medical field. More validation in clinical trial is required on the value of liquid biopsy in the cancer field to support the clinical utility of the test. There's also more studies needed to assess the tumors to the test accuracy and ability to identify risk tumor types. It is not clear that whether liquid biopsy provides a representative sampling of all genetic clones within a tumor or if there's a bias to specific sub regions of the tumor. There's still some challenging in the test sensitivity given the circulating tumor DNA or circulating tumor cells are relatively still very low in the circulation because most of the circulating DNA are from the normal tissue.

**Dr. Maria Arcila:**

Yeah, I think that perhaps the highest disadvantage of liquid biopsies is that a liquid biopsy and the ability to detect a mutation or a genetic alteration is highly dependent on how much DNA the tumor is shedding and different tumors may shed at different rates, and there are many variables that dictate how much tumor you're actually shedding free into the circulation. And this has to do with the amount of vascularity of the tumor. This has to do with the amount of necrosis, how close it is to a vessel, and even the renal function status of the patient, the exercise of that day and many patient related factors that really change how accurate and how informative that biopsy is going to be. A lot of these factors are still unknown and there are pre-analytic factors that may not be necessarily known to the individual that is testing the tissue.

So with this high amount of variability, the most important thing to remember is that you may end up with a false negative. Well, the advantage would be that if it is positive, you will spare the patient from a biopsy. If it is negative, you just do not know that it is actually negative. Now the other thing is that in a liquid biopsy, you are testing for mutations and mutations, it may overlap between diseases. So the fact that you have a mutation will tell you that you may have a disease, but it doesn't tell you what disease you have, so it's not diagnostic.

**Lisa Tomcko:**

Wow. Thank you both so much for taking us through those advantages and disadvantages of liquid biopsies. And just a follow up question, what would you say are the leading areas for liquid biopsy application at this present time?

**Dr. JinJuan Yao:**

For the current heart area for liquid biopsy application, I think the biggest area is for the monitoring of drug treatment or early detection of relapse recurrence. So there's a new concept where monitoring the treatment with the concept of minimal residual disease or molecular residual disease. With this kind of liquid biopsy, we're able to detect the presence of the prior detect alterations from the tissue. We can use that as a biomarker to see, to dictate the treatment response of the patient post either targeted therapy or chemotherapy. I think that's a very big application. So they don't need, maybe this is more sensitive than the imaging, so they can spare the CAT scan, which can only detect when the size of the tumor reached to a certain point. But the liquid biopsy, because of a sensitivity, lower limited detection can be as low as 0.1%. With current technology, it is more convenient also to monitor the patient with multiple liquid biopsy in the disease process. Another hot area maybe in the future will be early detection with methylation profile and with the fragment size of the CT DNA, there's a possibility we can detect cancer earlier in the bloodstream when there's no clinical presentation or no imaging changing yet. So because of the different cancer may have different methylation profile and the size of the fragments also going to be different. So combine all this also in using the AI technology with assistance of all this, then we may be able to find the cancer at the early stage.

**Dr. Maria Arcila:**

Yeah, I think that just to add to what Dr. Y was saying, there are obviously many opportunities and areas where we would like liquid biopsies to be leading. I do feel that at this point, the highest opportunity and the most immediate is really the detection of targetable biomarkers, which you can do rapidly because you cannot, sometimes you cannot schedule the patient for a biopsy or you don't have enough tissue to test, or you may have to go ahead and do another biopsy because the initial biopsy may not have been diagnostic. So by using liquid biopsy, and if you are able to detect a biomarker that is targetable, then you have that immediate opportunity. I think that most of what we see right now, even though the intent is really this monitoring, the most readily applicable aspect of liquid biopsy is the diagnosis of a biomarker.

**Lisa Tomcko:**

It sounds like a lot of promising potential for liquid biopsies as a technique for, as you say, both monitoring of already detected diseases and then potentially early detection of diseases and cancers. And where have you seen the greatest opportunity or application in your practice?

**Dr. Maria Arcila:**

So in our practice, the greatest application at this time is in the ability to screen for a targeted biomarker. This can be done rapidly because sometimes the patients may come from another institution where getting the tissue may be difficult. The transfer of those samples to our institution to be able to test, sometimes it can take a long time and then you can triage the patient very rapidly if they have advanced disease by doing a liquid biopsy while you wait for that tissue. But clearly I think that sometimes you actually need both. As I said before, sometimes they could be negative, so you have to do both the cell-free DNA and the tissue testing. I think that while the current focus has been predominantly on that detection of cell-free DNA from plasma, we have seen equal exciting opportunities with cell-free DNA from other body fluids. For instance, our institution right now is the only institution doing the clinical testing of cell-free DNA from CSF.

And this has been a game changer for the assessment both primary and metastatic tumors to the central nervous system because the central nervous system is a highly protected area. The brain has a blood-brain barrier that doesn't allow tumors in the brain to be detected easily in peripheral circulation. Then by doing a liquid biopsy of the CSF, then you have a great opportunity to be able to detect mutations that are specific to that area. So within the central nervous system, for example, tumors may develop clonal evolution or resistance mechanisms that are absolutely unique to the brain because of that blood brain barrier. So testing that liquid biopsy is not only very valuable, but is unparalleled by any other sampling or testing technique. Similarly, the use of cell-free DNA testing and liquid biopsies in body fluids in other body fluids is equally important for lung cancer. For example, you may end up with patients that may not be amenable to a biopsy, but they have a pleural effusion where you have cell-free DNA that you can test.

And this also is applicable to cytologic procedures where sometimes through doing an FNA, fine needle aspiration, you're actually damaging cells and you have DNA that is released from the cells and in the fluid of that cytology and washes of a brush procedure, you can actually get an enormous amount of cell-free DNA that you can test. And this is the type set of DNA that has been discarded in laboratories because we didn't know that that was where you had a lot of DNA. But now this is actually being applied not only at our institution but in many other institutions. So the use of cell-free DNA from supernatant from washes is very valuable. I think of interest as well. There are body fluids like urine, and while you may think of urine as a body fluid where you would test something that is specific to the bladder or to the region, we have actually published on pediatric patients detecting BRAF mutations from urine from his osteolytic neoplasms. And this is because in a pediatric patient, you will not be able to get a lot of blood to be able to get a plasma and a cell-free DNA to test, but you can actually collect a lot of urine in this patient. So there are great opportunities.

**Lisa Tomcko:**

That's so exciting to know that liquid biopsies are expanding to other bodily fluids and giving us insights into those parts of the body and what's going on with 'em. Dr. Yao, did you want to say anything additionally?

**Dr. JinJuan Yao:**

Yeah, I just want to discuss in the CSF ctDNA is highly concentrated or enriched there sometimes, many times actually we see that ctDNA testing in CSF is a priority, but the cytology is completely negative. I think it is a great additional information, very important. We can use the ctDNA testing from CSF to provide the clinical team additional information to manage that patient.

**Dr. Maria Arcila:**

Yeah, that is actually correct, and it's a great point because sometimes because of this blood brain barrier that you have across the central nervous system, you don't have contaminating DNA from normal cells. So you could even detect mutations that variant allele frequencies that are incredibly high where you wouldn't need special techniques that are highly sensitive to actually test them. So it presents a great opportunity for the people or for the institutions that have highly sensitive assays, but you could even use standard assays of regular sensitivity to be able to test these samples.

**Lisa Tomcko:**

And are there any practical suggestions for pathologists on how to implement the liquid biopsy methods successfully into their practice?

**Dr. Maria Arcila:**

I think that the most practical suggestion that I would have is for pathologists to stay informed and up to date. The thing about liquid biopsies is that the science is rapidly emerging and the technology is swiftly being adopted in clinical care because there is an unmet need. If you don't have a tissue to test, then the liquid biopsy is the next best thing and sometimes a better thing while you wait for that tissue. At this time, the guidelines are just being published. So it has been a long time waiting for these guidelines, and there are still a lot of things that are not answered. But just to highlight that, just two weeks ago, the Association for Molecular Pathology and the College of American Pathologists published the first recommendations for validation of cell-free DNA as a joint consensus recommendation. And I would just refer the listeners for this amazing resource for validation, implementation and information on the pre-analytic aspects of testing.

This also covers collection protocols and additional resources. So where I think that it is really important is to ensure that while you do not know a lot of the pre-analytic variables, such as the patient specific factors and the tumor specific factors, the laboratory should be very stringent about their collection protocols. We, for example, utilize strep tubes for all of the cell-free DNA samples to that the sample remains viable and not contaminated by normal DNA from blood cells. And then that means that with this type of collection tube, you have sufficient time to wait in case something happens to that sample. So the stability is several days as opposed to EDTA tubes, for example, where the stability is just a few hours and starts degrading very soon after the first day to recognize what are the limitations of the specific laboratories, how long would it take you to get to that sample? Are you batching? So to understand the process and recognize the limitations that you have to preserve that tissue as much as you can.

**Lisa Tomcko:**

Good to know. The CAP has put out recommendations on validation of cell-free DNA assays willing to that in the show notes. And where do you think this technique of liquid biopsying will go in the next five years to make the biggest impact on patient care?

**Dr. Maria Arcila:**

Yeah, I think that currently the bulk of the application of liquid biopsies in the clinical space is really confined to the rapid detection and the intent to monitor the patients for that specific marker that you detected in the sample. But I think that in addition to the emerging application of treatment response, residual disease monitoring and emergence of resistance mechanisms, there are many, many other opportunities. There is more fine tuning of the technology to be able to diagnose better. So one of the issues is that finding a mutation does not specifically inform you what the primary diagnosis is because the mutation profiles may overlap significantly across different malignancies. So the fact that I would detect a KRAS mutation doesn't mean that the patient will have a colon cancer or a lung cancer. You actually have to find out exactly what is it that the patient has making full use of additional components of the liquid biopsy.

It is very important, and I think this is where the science is going at this time, doing DNA methylation patterns and profiles, which could potentially inform what the type of tumor is, is something that is still very much in the research area, but that would make the hugest impact in the clinical space. Analyzing the DNA fragment sizes, analyzing RNA, circulating tumor cells, extracellular vesicles, exosomes, and even tumor educated platelets would be of high value to be able to truly use this technique beyond the marker ability to be able to not only detect disease, understand what type of disease, diagnosed disease at early stage, which is not something that can be done right now because of the limitations that we talked about before, but bringing this from the research space to the clinical space is going to take a while. So I do think that fine tuning the techniques and understanding how this can actually help together, not just for a single combination of technologies on this type of template would have the biggest impact in patient care.

**Dr. JinJuan Yao:**

So I also get your opinion regarding how do you're going to choose D-D-P-C-R versus large panel NGS testing?

**Dr. Maria Arcila:**

Well, it really depends on what the intent is for the assay, but I do feel that because a lot of what we're doing really depends on multiple markers, you really need NGS technology. I think that up until this point, we have been thinking about known resistance mechanisms. So for instance, there are guidelines about being able to use digital PCR for T790M mutations or EG FR T790M mutations as the most common mechanism of resistance for low cancer patients with EGFR mutations and who have been on an EGFR inhibitor. But the truth of the matter is that now we have moved beyond the first generation EGFR inhibitors where you are now treating the patients with third generation inhibitors, and now they have new resistance mechanisms, which are not only mutations, but they are actually fusions and other resistance mechanisms that you wouldn't be able to detect with a digital PCR assay.

I think that some people may want to say, well, you could test with a panel by digital PCR, but again, the truth of the matter is that you usually don't have a lot of DNA that you may recover from a liquid biopsy to be able to do multiple tests in tandem or at the same time. So I do think that the space is really for NGS to be able to do the testing and to be able to broadly capture the biology of that tumor of what is happening to the patient. Again, we are now thinking not only about tumor markers and mutations for instance, but there is a lot of research and understanding the microbiome and recognizing that some diseases have a specific microbiome that is associated with those diseases. So now you're not only capturing the tumor, but you're capturing all of the biology of what is going on with the specific disease process, and not only for a disease process, but for non neoplastic applications as well.

**Dr. JinJuan Yao:**

Yeah, I totally agree. I think tumor informed, and also this has some kind of implication with the approach tumor informed or tumor agnostic. So for the tumor informed, do we need to design specific each panel for each individual patient? What are the regulation? Do we have to validate each single of those tumor informed assay?

**Dr. Maria Arcila:**

That's actually a great question. I mean, the issue of tumor informed assays is that obviously you are doing the profiling in the tissue initially, and then you're looking specifically for those mutations in the cell 3D NA and creating a panel that is just specific to that tumor. I think that's a great approach to follow the unique markers that were there initially. And then of course, that really empowers your minimal residual disease assessment because you can do it with better sensitivity. But at the end, these tumors, and especially if you have patients on targeted therapies, they're going to develop resistance mechanisms or sub clones that are part of the clonal evolution of that tumor. And if you're not including all of the other markers in the panel, then you really won't be able to know that there is evolution of that tumor. So I think that the applications are really endless. So I believe that if you're going for for the highest sensitivity, then you really have to enhance the detection of those specific markers, but confining yourself to only those markers will really decrease the value of your assay for the additional markers of that tumor as the tumor evolves.

**Lisa Tomcko:**

Well, it sounds like there's still a lot of untapped potential remaining in the liquid biopsy space, so that's exciting to think about what's on the horizon potentially. And finally, any concluding thoughts?

**Dr. Maria Arcila:**

I think that the most important thing about liquid biopsies and utilization of liquid biopsies at this point is the fact that this actually marks a new beginning, a new paradigm in diagnostics. Many of us pathologists may be not entirely happy about. I think that pathology in general has been such that we think of us as pathologists, as the people who follow the tissue, who know the tissue, and who know the biology based on the morphology. But this is no longer the case. So as a pathologist whose practice is primarily based on morphology, this change really may lead one to believe that there is going to be a decreasing role for the pathologist as we know it. But what this actually does is just that brings an entirely new facet to the role of the pathologist that we have to get ready for and we actually have to embrace it as such. So education, finding your role in this new science as a pathologist is particularly important because at the end of the day, the pathologist has a very unique understanding of disease that other physicians do not have. So it is critical that we remain in the central role for shaping the science and ultimately delivering the appropriate care of the patient. And the pathologist has the attributes that one would need to do that, but we do have to embrace the new technology,

**Dr. JinJuan Yao:**

The future of the integration between tumor tissue and liquid testing. So I think that's because the tumor is becoming a chronic disease. Think in the disease process, we need continuously monitor the patient by which the tissue probably will not be that we cannot biopsy patient multiple times, but we can draw blood that's application for this technology.

**Lisa Tomcko:**

Well, a big thank you to both of you, Dr. Arcila and Dr. Yao for the great discussion on liquid biopsies. And a big thank you to our listeners as well. You can learn more about liquid biopsies and other precision medicine topics by going to the Precision Medicine Resource Center on the CAP's website. The link is in the description. You can also find the link to the recommendations on validation of cell-free DNA, assays from the College of American Pathologists, and the Association for Molecular Pathology. Stay tuned for future episodes of CAPcast and for more information from the CAP visit cap.org.