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Liquid Biopsy Hotspot Variant Assays: Analytical Validation for Application in Residual Disease Detection and Treatment Monitoring

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Guest: Ariane Hallermayr is a researcher at the Medical Genetics Center and PhD candidate in Medical Research in Epidemiology and Public Health at the Ludwig-Maximilians University in Munich.

Bob Barrett: This is a podcast from *Clinical Chemistry*, sponsored by the Department of Laboratory Medicine at Boston Children's Hospital. I'm Bob Barrett.

Analysis of circulating tumor DNA and plasma is a powerful approach to guide decisions in personalized cancer treatment. However, the concentrations of circulating tumor DNA are low in plasma and highly sensitive methods are required to reliably identify clinically relevant variants. But which of the assay techniques provides optimal sensitivity yet minimizes false positive signals? A paper appearing in the November 2021 issue of *Clinical Chemistry* may help to address that very question. That paper evaluated the performance characteristics of five droplet digital PCR assays to detect and quantify pathogenic variants of three key oncogenes. The lead author for that study is Ariane Hallermayr, a researcher at the Medical Genetics Center and PhD candidate in Medical Research in Epidemiology and Public Health at the Ludwig-Maximilians University in Munich. Her research focuses on the potential of circulating tumor DNA analysis for monitoring patients with solid cancers and implementation into clinical practice. She is our guest in this podcast. First of all, what are the most important advantages to patient care that can be brought about by using circulating tumor DNA as biomarkers?

Ariane Hallermayr: So, traditionally, molecular pathological diagnosis is performed based on tissue biopsies and there are unfortunately or probably well-known limitations regarding this approach. Since usually highly invasive approaches are required to obtain tumor tissue and therefore it is not possible to frequently analyze those tissues. And also, especially under treatment, it might not be possible to obtain all required information at a specific time point to support or to guide the course of treatment. Further, tissue biopsies represent only a distinct tumor side rather than the complete tumor heterogeneity and therefore, it might not be possible to get access to all information required for diagnosis and treatment decisions at a certain time point.

Currently, a widely discussed alternative or addition to tissue biopsies are liquid biopsies and circulating tumor DNA. The marker we discussed in our paper is a fraction of whole circulating free DNA and it's an aniline present in liquid biopsies. And in contrast to tissue biopsies, liquid biopsies can be easily obtained by simple blood withdrawals and therefore, circulating tumor DNA analysis is applicable for real-time monitoring in cancer patients and further circulating tumor DNA is released from any tumor side and therefore the complete tumor heterogeneity, including primary tumor and metastasis is involved.

Bob Barrett: You just talked about the advantages of circulating tumor DNA in a more general setting. How could this analysis provide relevant information in clinical practice?

Ariane Hallermayr: So, in clinical practice, actually, circulating tumor DNA analysis may be used as an innovative tool and companion diagnostics for the detection of genetic tumor biomarkers. As mentioned earlier, it overcomes the limitations known about tissue biopsies and there are even current clinical practice guidelines, for example, for advanced non-small cell lung cancer or advanced breast cancer that recommend genetic testing on circulating tumor DNA to guide therapy decisions, which is especially very good or yes, the case where no tumor tissue is available. And then analyzing circulating tumor DNA can support decision-making for clinicians. And in addition to companion diagnostics, there are plenty of studies indicating that detection of circulating tumor DNA following a surgery represents molecular residual disease and patients with molecular residual disease are actually more likely to develop disease recurrence. Then there are even more studies showing or indicating that circulating tumor DNA levels correlate well to tumor burden in cancer patients, and therefore, quantification of those circulating tumor DNA levels could serve as an indicator for disease progression in cancer patients.

Bob Barrett: Best to quickly follow-up in your study in *Clinical Chemistry*, you focused on three oncogenes. Can you tell our listeners what they are and why did you select those?

Ariane Hallermayr: So, the three oncogenes we selected in our study are EGFR, KRAS and BRAF.

EGFR is actually one of the most important oncogenes in non-small cell lung cancer as hotspot variants and this gene can be used to indicate targeted therapies. Then there are also BRAF and KRAS which are oncogenes very important also in non-small cell lung cancer but also in colorectal cancer or for example, melanoma. So, in case of BRAF, the BRAF gene, there might be one target variant, especially pV600E variant, which can be targeted by MEK and BRAF inhibitors. And the

KRAS gene are hotspot variants and the KRAS gene might be contraindicators for EGFR targeted therapies.

Bob Barrett: Circulating tumor DNA has the potential to provide important information for non-invasive follow-up in cancer patients. Why is this specific application of circulating tumor DNA analysis not yet widely implemented into clinical practice?

Ariane Hallermayr: So far, in my opinion, specific guidelines for clinicians are missing. How a circulating tumor DNA analysis results should be interpreted, therefore, can be used to guide, for example, therapy decisions. In most cases, current circulating tumor DNA assays are validated with a focus on the limit of detection as a parameter for sensitivity. And the limit of detection is, of course, very important to be aware of lower circulating tumor DNA levels, which might not be detected, but still, it cannot support the interpretation of circulating tumor DNA analysis results as it does not represent the real critical cut off. And rather than focusing only on the sensitivity of circulating tumor DNA assays in a clinical setting, especially the specificity is critical as false positive results might lead to overtreatment. And further, if circulating tumor DNA analysis should be applied for disease monitoring, validation of the quantitative range of an assay, including reliable confidence intervals is crucial.

Bob Barrett: So, some clinicians may remain insecure regarding the interpretation of circulating tumor DNA analysis results. What's needed for reliable interpretation of these types of laboratory results?

Ariane Hallermayr: As mentioned earlier, actually, the specificity of circulating tumor DNA assay is critical as it represents a critical cut off to distinguish between circulating tumor DNA positive and negative results, meaning the presence or absence of circulating tumor DNA in a patient sample. And this cut off can be -- this actually the limit of Planck. Therefore, the limit of Planck is the cutoff for specificity to distinguish between true positive and true negative results. In a clinical setting, the limit of Planck is very important, for example, for the detection of molecular residual disease after surgery, but also for companion diagnostics to guide therapy decisions since only lowest levels of circulating tumor DNA might be present. And in addition to the limit of Planck, also the limit of quantification is a critical parameter for clinical interpretation and it serves as a clinical cut off kind of, since only circulating tumor DNA levels above the limit of quantification can be quantified with a reliable confidence interval. Therefore, it's important when interpreting, for example, decreases or increases in circulating tumor DNA levels as an indicator for a response or resistance to treatment.

Bob Barrett: Well, finally, in your opinion, what information should be provided on laboratory analysis reports of circulating tumor DNA to allow robust clinical interpretation?

Ariane Hallermayr: Yeah. In my opinion, I would say that it is important to provide all specific information on performance metrics of the assay used and this would be, first of all, the limit of detection which is currently used for most circulating tumor DNA assays as the parameter for sensitivity. Since for clinical interpretation, it is critical to know that circulating tumor DNA levels below the limit of detection will not be reliably detected. Further, as mentioned earlier, the limit of Planck should be included to ensure that clinicians know that only true positive results are included in the report and that every -- on the lowest levels of circulating tumor DNA which are reported are actually true positive results.

And then, especially when a clinician intends to perform or use circulating tumor DNA analysis in a treatment monitoring setting, circulating tumor DNA levels should only be quantified above the limit of quantification, specifying the respect of confidence interval because this then ensures that interpretation regarding response or resistance to treatment is only performed in a quantifiable range of an assay. Otherwise, results might not be reliable. But finally, I'd also like to emphasize that circulating tumor DNA is not released from each tumor and therefore, negative circulating tumor DNA analysis results do not automatically indicate the absence of tumor. It might just be that the tumor does not release circulating tumor DNA, therefore, circulating tumor DNA cannot replace current clinical diagnostic tests, but rather etched probably an easy accessible biomarker to patient care.

Bob Barrett: That was Ariane Hallermayr from the Medical Genetics Center and the Ludwig-Maximilians University in Munich. She has been our guest in this podcast on accurate tumor burden analysis in liquid biopsies. She is the lead author of a paper on the analytical validation of circulating tumor DNA analysis that appears in the November 2021 issue of *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.