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RNA Profiles of Circulating Tumor Cells and Extracellular Vesicles for Therapy Stratification of Metastatic Breast Cancer Patients

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Guest: Corinna Keup is a researcher at the University Hospital of Essen, Germany, in the Department of Gynecology and Obstetrics.

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

Liquid biopsy methods are an exciting area in cancer screening, diagnosis, and management. These methods are aimed at measuring genomic signatures of tumor cells in the blood. They rely on detection of circulating tumor cells, tumor-derived extracellular vesicles, or exosomes, or cell-free circulating DNA. Liquid biopsy methods have been proposed to compliment, enhance or perhaps even replace tissue biopsy in some scenarios.

Beyond the minimal invasiveness, performing liquid biopsy may overcome some other weaknesses of tissue biopsy that are related to a single site's inability to capture intratumor heterogeneity or be representative of all the changes within the tumor. All three approaches to detect a tumor's genetic material have been applied for therapy stratification and monitoring in breast cancer. However, knowledge about the differences between these methods in the same patient cohort is limited.

An original article in the July 2018 issue of *Clinical Chemistry* compared messenger RNA profiles of circulating tumor cells and extracellular vesicles in patients with metastatic breast cancer to estimate the utility of each in therapy management.

For this podcast, we're joined by the article's first author, Corinna Keup. She is a doctoral researcher at the University Hospital of Essen, Germany, in the Department of Gynecology and Obstetrics.

So the first question, what was the overall goal of this study and what's special about this work?

Corinna Keup: All right. We aimed to compare the mRNA profiles of CTCs and EVs, so circulating tumor cells and extracellular vesicles of matched samples, since no one ever compared these two fractions, and we correlated the overexpression signals in

both fractions to the clinical staging result and to therapy regimens to identify prognostic, predictive, and monitoring markers.

And special about the presented work is that we have a quite clearly defined cohort of metastatic breast cancer patients with hormone-receptor positive and HER2 negative primary tumor. In fact, the evaluation of the HER2 encoding gene, ERBB2, is quite stringent in this cohort.

And we found interestingly that ERBB2 overexpression in CTCs was found in 40% of all patients of this cohort. And this finding highlights the intratumor heterogeneity since we found out that CTCs, and these are the treated minimal residual diseases, show a disconcordance HER2 status compared to the surgically removed breast cancer primary tissue.

And the patients with the ERBB2 overexpressing CTCs showed a decreased therapy response, so this might be a monitoring strategy. And moreover, they might benefit from an anti-HER2 treatment, so the ERBB2 transcript and CTC might have a predictive value as well.

Bob Barrett: So, why had no one compared these two components and what types of difficulties did you face while you were doing it?

Corinna Keup: Well, the variants found in tissue DNA and cell free DNA were intensively compared in the past. And there is even a growing number of comparison studies of genomic DNA and cell free DNA. But the transcriptional profiles of CTCs and EVs were not compared previously. And this might be due to the low RNA concentration in EVs, but also the challenging low number of CTCs. And our method included the preamplification of the cDNA previous to the qPCR and we excluded any quantification of the RNA or cDNA upfront of the qPCR.

And another critical aspect of our project is the isolation technique of the EVs. For the EV fraction without contamination, we excluded any precipitation on an ultracentrifugation method, but we used an affinity based method for binding all subgroups of EVs to a column by exploiting their common surface properties. Thus we however, isolated not only tumor-derived EVs but also the EV secreted from, for example, immune cells, since we normalized all signals to healthy controls however, only the tumor-associated EV signals were found. However, the included tumor-associated EV signals gave additional information about the immune cells that are involved in the tumor-induced processes.

Bob Barrett: How will the results of this study move the liquid biopsy field forward?

Corinna Keup: We showed that the transcriptional characteristics of CTCs and also EVs are promising markers for prognostic, monitoring, and therapy stratification in metastatic breast cancer. And our complimentary results of the CTC and EV fraction however, warn against extrapolation of any value of biomarker result for the translation into clinical practice.

Bob Barrett: Will we soon see extracellular vesicles or circulating tumor cell messenger RNA profiles validated for clinical use?

Corinna Keup: Liquid biopsies are definitely promising for therapy monitoring. For breast cancer therapy stratification, CTCs are already included in clinical trials. And any first successful trial will push the CTC analysis into clinical practice.

In this regard, I believe it is important not only to quantify the CTCs, but also to characterize the CTCs to get clinically relevant insight. Until now the EVs are not tested commonly in clinical trials. And there is still a debate about the most usable isolation technique for CTCs.

Bob Barrett: What are the challenges that you think you'll be facing in the future with this work?

Corinna Keup: The discrepancies of CTC isolation methods are still tensioning the field, so common consensus has to be achieved in the future. And moreover, in the primary setting of, for example breast cancer, CTCs are quite rare. Thus, the implementation of this analyte might be challenging in terms of the demanding sensitivity needed.

Bob Barrett: Let's look ahead. Where do you see this work in five or even ten years from now?

Corinna Keup: In the metastatic setting, CTC detection in breast cancer patients is not a rare event, enabling the CTCs to be a useful marker in the clinic. And in my opinion, this implementation will be successful in the near future, having the advantage that therapy decisions based on the characteristics of the minimal residual disease rather than only on the primary tissue characteristics that are invasively assessed and they don't mirror the whole tumor heterogeneity anyway.

And considering the growing interest in the immune checkpoint inhibitors, the analysis of the EV profiles, and these also include the EV secreted by the immune cells, the EVs might be promising in the future everywhere.

Bob Barrett: Corinna Keup is a doctoral researcher at the University Hospital of Essen, Germany in the Department of Gynecology and Obstetrics. She has been our guest in this podcast from *Clinical Chemistry*. I'm Bob Barrett. Thanks for listening.