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# Potential of liquid biopsy in diagnosis and monitoring of malignant invasion

Elena Pacheshkoska, Marija Hiljadnikova-Bajro

*Faculty of Pharmacy, Ss. Cyril and Methodius University in Skopje, Mother Theresa 47, 1000 Skopje, Republic of North Macedonia*

## Introduction

Detection of cancer in the early stages is crucial for its effective treatment and favorable prognosis. Hence, a lot of efforts are being invested in identifying novel methodologies with low invasiveness, high specificity and sensitivity, to be employed in the diagnosis and monitoring of malignant invasion. One of latest achievements in this field is the liquid biopsy, which current status is reviewed in this article along with its potential use in diagnosing and monitoring colorectal, lung, melanoma, prostate, ovarian, bladder, hepatocellular, central nervous system, breast and pancreatic cancer. Liquid biopsy is not yet established in the routine clinical practice, but recent research suggests its potential to become the gold standard in oncology diagnostics in the near future.

## Liquid biopsy - a promising novel methodology in the oncological laboratory

Liquid biopsy is an innovative, promising technique able to comprehensively characterize a malignant disease in a minimally invasive way utilizing samples of blood, saliva, urine which are much easy obtained in comparison to tissue specimens. Furthermore, the short processing time, low failure rate and high tolerability, make this technique applicable for monitoring of the treatment response (Rodríguez et al., 2021). A variety of analytes can be identified in the patients' samples including: circulating tumor cells (CTC) and circulating tumor DNA (ctDNA), circular RNAs-in particular microRNA (miRNAs), circulating transcriptome which represents different long non-coding RNAs (lnRNAs) and coding RNA, autoantibodies, exosomes and other extracellular vesicles, that have gained tremendous interest in the scope of biomarker research.

CTCs provide relevant information on the tumor composition and invasiveness and have the potential to determine the effectiveness/need of a specific therapy and guide the therapeutic treatment. They can be detected even in absence of metastases and provide information on the mechanism of drug resistance. On the other hand, ctDNA can more accurately represent the existence of the tumor in real time and the likelihood of recurrence in patients receiving therapy. Furthermore, ctDNA can originate from any tumor lesion in the body, so that sampling bias is minimized, it enables genotyping and thus the use of personalized, targeted therapy but also can be used for dynamic monitoring of the cancer progression via the genetic mutations associated with different stages of the cancer development. Exosomes are used as diagnostic analytes because they carry the protein, DNA, and RNA transcript making them a small set of biomarkers with multiple analytes (Yu et al., 2021). Analysis of ctDNA methylation can enable the diagnosis of high-risk patients with HCC (hepatocellular carcinoma) at an early stage and also become an alternative method of HCC monitoring in the future. The molecular characterization of ctDNA and CTCs can be applied for more advanced monitoring of the post-treatment CTC levels which indicate tumor recurrence and reduced survival in patients, while the exosomal miRNAs allow early diagnosis of HCC. CTCs test is also used as a prognostic tool for breast, prostate, pancreatic ductal adenocarcinoma and colon cancer. Furthermore, miRNA can act as a tumor suppressor and thus stop tumorigenesis but on the other hand can lead to metastatic progression and cancer growth. Several studies have found that miRNAs can be detected in serum, mononuclear cells,

*ele-paceskoska@hotmail.com

and whole blood in cancer patients, including miRNA-195 and let-7a in breast cancer, miR-21, miR-210 and miR-486-5p in lung cancer and miR-141 in prostate cancer (Yang et al., 2022).

Exosomes have also been shown to contain long non-coding RNAs (lncRNAs), which act as messengers of interaction between two cells, some lncRNAs being more abundant in exosomes while others are less common. Recent research has shown that exocosome-derived lncRNAs affect tumor apoptosis, proliferation, and migration, but is also involved in angiogenesis promotion. A biomarker of this type, that has already been approved for clinical use in diagnosing of prostate cancer is PCA3, but many other LncRNAs could be associated with malignancies like the thyroid, bladder, liver, lung, gastric, esophageal, colorectal, breast cancer and gliomas (Preethi et al., 2022).

Circular RNAs (circRNAs) have greater diagnostic and prognostic accuracy for several tumor types and the ability to monitor their progression, but they are still under development with great potential to be applied in clinical practice in near future (Wang et al., 2021). The location of the primary or metastatic lesions affects the presence of circular RNA in various body fluids. Circular RNA as a potential biomarker has been tested in a number of cancers including gastric, pancreatic, breast and small cell lung cancer, hepatocellular, colorectal and urothelial bladder carcinoma, lung adenocarcinoma and chronic myeloid leukemia. CircRNA is a stable biomarker with high tissue specificity. So far, several circRNA molecules have been evaluated for their potential to be used as diagnostic biomarkers. Several of them, including Cdr1as, circITCH, circPVT1, and circHIPK3, have been expressed in a variety of cancers. Cdr1as (ciRS-7) is a regulator of miR-7 circRNA, a tumor suppressor in breast, lung, and brain cancer. Some circRNAs also enable a differential diagnosis between cancer subtypes, such as circPVT1, that is associated with a favorable prognosis in gastric cancer and a poor prognosis for colorectal cancer.

Among the liquid biopsy biomarkers in the focus of latest investigation is the enzyme carbonic anhydrase IX, enzyme for diagnosing and monitoring cancer progression. Namely, tumor cells use specific reactions catalyzed by the carbonic anhydrase to lower the extracellular pH by producing lactic acid and hydrating $CO_2$, involving thereby the carboanhydrase in tumor growth and development. CA IX is markedly expressed in solid tumors of the uterus, kidneys, lungs, colon, breast, brain, and ovaries (Ozensoy Guler et al., 2020).

## Conclusion

The data presented clearly emphasize the emerging potential of liquid biopsy to be employed as a minimally invasive, precisely tailored and cost-effective method in clinical diagnosis and monitoring of malignant invasion. Clinical application of liquid biopsy is already paving the way for precision theranostics and personalized medicine. The most investigated and advanced biomarkers for liquid biopsy are circulating tumour cells (CTCs), circulating cell-free tumour DNA (ctDNA), circular RNAs (circRNAs), micro-RNAs (miRNAs), long non-coding RNAs (lncRNAs), tumor-derived extracellular vesicles (tdEVs), and exosomes. All of them contribute to define the levels of disease progression and meet the two main criteria of good biomarkers that are being representative of all the neoplastic lesions that a patient has at a certain point of time and allow serial longitudinal analyses with minimal or no discomfort for the patient.Therefore, it is anticipated that different approaches in the liquid biopsy will revolutionize the clinical practice by offering novel insights into the precision medicine of oncology.

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