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Popular Article

Liquid Biopsy: New Era of Game Changer Diagnostic Tool for Veterinary Oncology

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Abstract

Liquid biopsy requires sampling of blood, urine and other body fluids rather than tissue to detect cancer and disease markers. This technology can detect DNA fragments released by tumors or proteins associated with various tumor types and widely used for cancer screening in humans and animals. Liquid biopsies will complement the tissue biopsy allowing more cancer patients to be tested. Liquid biopsy tests do not replace gold-standard diagnostic methods such as tissue biopsy; rather, they offer a complementary approach to the identification and monitoring of cancer patients. This article explores the diagnostic capabilities of liquid biopsy tests in veterinary medicine.

Key word: Blood, cancer, biomarker, future diagnostic techniques

Introduction

A liquid biopsy is an easy, simple and non-invasive diagnostic method to identify and monitor cancer or disease through/via biomarkers (Bao *et al.*, 2024). It's alternative to surgical biopsies which enables doctors to discover a range of information about a tumors through a simple bio-body fluids such as blood, saliva, urine, cerebrospinal fluid, and pleural effusions and other body fluids (**Fig. 1**). Traces of the cancer's DNA in the blood can give clues about which treatments are most likely to

work for that patient. The non-invasive nature of liquid biopsies, which require only 5 milliliters of blood, means they are much easier to tolerate and the procedure is quicker than a surgical biopsy. The blood sample is then 'spun down' to get 2 milliliters of plasma which can be analyses for the tumour DNA.

Most cancers have multiple genetic mutations and they may not have the same in all parts of the cancer. The tissue samples removed for biopsy may not show all mutations whereas liquid biopsies offer an improved chance of detecting these genetic changes. The benefits of non-invasive, quick and easily repeatable tests are clear. And in the longer term, liquid biopsies may ultimately be used to catch signs of cancer early, before symptoms arise. This could make a significant difference to the way we understand and treat the cancer."

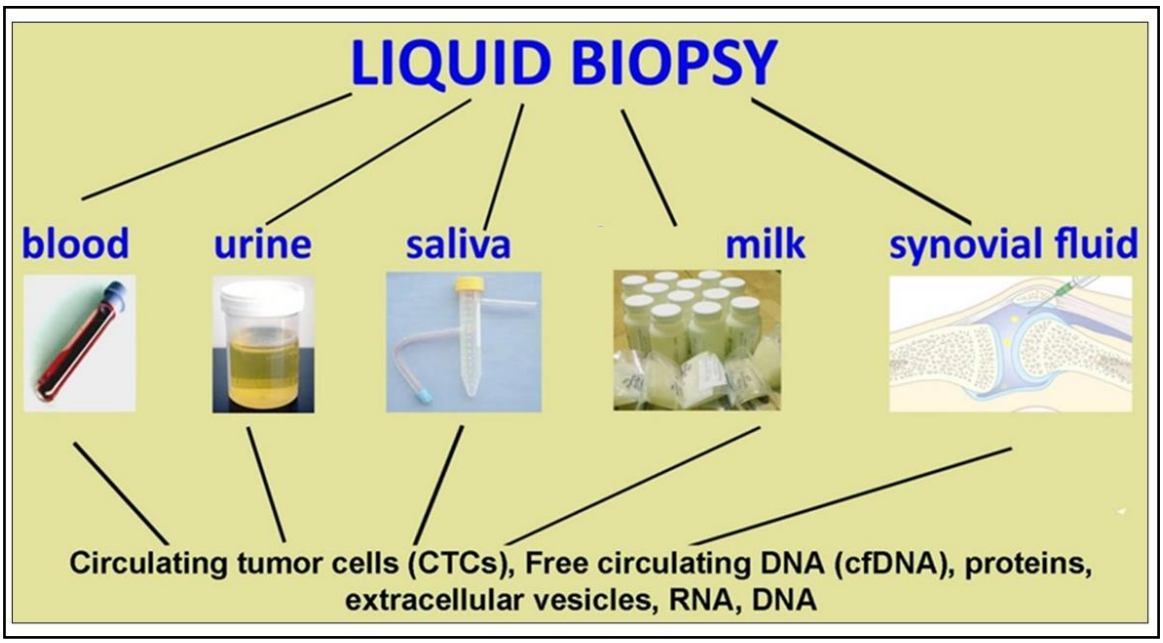

Figure 1: Various body fluids for identification of tumors induced molecule for early cancer diagnosis

Is This the End Of Tissue Biopsies?

Liquid biopsies have the potential to revolutionize of cancer care. However, there are challenges to the widespread use of this new approach and there is still a place for tissue biopsies. Far from being redundant, taking and testing tissue samples remains the gold standard.

For liquid biopsies, it is still relatively early days and some questions still need to be answered. For example, to what extent does test accuracy vary among tumour types and stages of disease? Does the liquid biopsy provide a representative sampling of all the genetic clones in a tumour or is there a bias to specific sub-regions? Researchers are currently working to answer these questions. Liquid

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biopsies will complement the tissue biopsy allowing more patients to be tested.

The liquid biopsy will not replace the tissue biopsy in the foreseeable future", Lifecycle Leader for Genomics and Oncology for Diagnostics. "However, liquid biopsies will complement the tissue biopsy allowing more patients to be tested. The issue is that many times there is just not enough tissue to test on. This novel, minimally invasive technique has the potential to change the prognostic and predictive landscape for lung cancer genotyping and impact patient management. Many potential molecular cancer precursors identified at early cancer stage (Bao *et al.*, 2024) and large number of information received through novel liquid biopsy like key biomarkers namely circulating tumor DNA, circulating tumor cells, microRNAs, and extracellular vesicles (**Fig.2**).

However, as scientific knowledge advances, researchers are learning more about the potential of liquid biopsies to detect mutations, suggesting that the promise and power of this diagnostic technology could be truly game-changing.

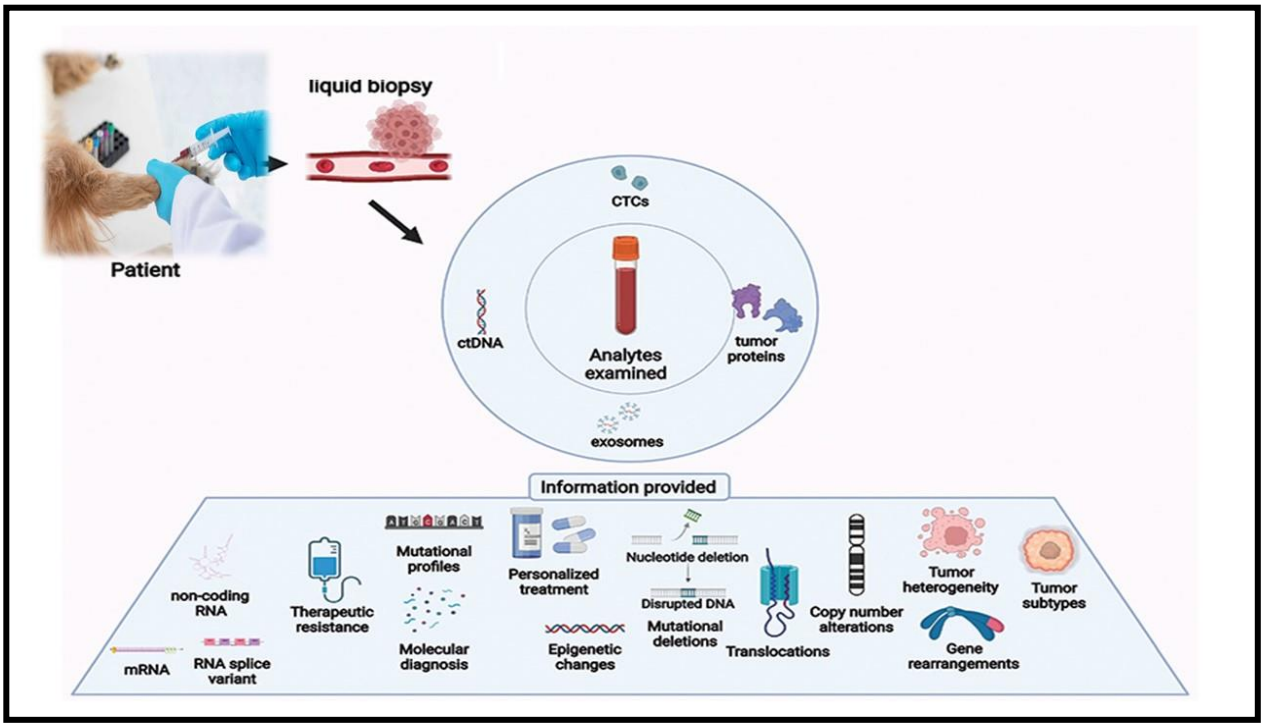

Figure 2: - Novel Liquid biopsy method for detection and identification of throughput-in-depth information for early cancer diagnosis

Clinical Application of Liquid Biopsy:

Mammary gland cancer: -

As the most common cancer in female animal, mammary gland cancer continues to be one of the leading causes of cancer-related deaths in worldwide. For the prevention, identification of the

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diseases at the regional stage and also, availability for the therapeutic programs (DeSantis *et al*., 2017). As a new and appealing option, liquid biopsy (which includes both CTCs (circulating tumor cells) and ctDNA (circulating tumor DNA)) has the potential to surpass some of the limitations of traditional biopsy and offer a more representative image of the heterogeneity of the disease. Liquid biopsy has demonstrated its potential for prognostic stratification, drug resistance identification, disease recurrence detection, and mammary gland cancer biomarker identification (Buono *et al*., 2019).

Rack et al. evaluated the potential role of CTCs as prognostic markers already at mammary gland cancer diagnosis for the stratification of patients needing adjuvant therapy in a sizable patient cohort. In this regard, Ma et al. discovered a strong correlation between the emergence of anti-HER2 therapy resistance and HER2-amplificated ctDNA. In addition, quantitative assessment, as previously mentioned, the detection of ctDNA mutations can be helpful in predicting treatment sensitivity.

Treatment resistance to aromatase (oestrogen synthase) inhibitors has been linked to the discovery of ESR1 (oestrogen receptor 1) gene mutations in ctDNA (circulating tumor DNA) from MBC (metastatic mammary gland cancer) patients (Andrè *et al*., 2020). With the recent approval of the **PI3K** inhibitor alpelisib (chemical compound to treat certain type of mammary gland cancer), liquid biopsy has become a clinically useful test in Mammary gland cancer.

Liquid biopsy has been used more frequently in mammary gland cancer as a result of the implementation of new treatment standards (PI3K and CDK4/6 inhibitors) to identify novel biomarkers of therapy resistance or response. In primary lesions, there was a strong correlation between the **PI3K** status and **Ki67**% expression. (Del Re*et al*., 2020)

Clinical application of PI3K for treatment of Advanced Mammary gland Cancer: -

The companion diagnostic test, there screen PIK3CA RGQ PCR Kit, detects the PIK3CA mutation in tissue or liquid biopsy. There are some other PI3K Inhibitors likely Alpelisib and buparlisib show efficacy and safety in treating mammary gland cancer, especially in PIK3CA-mutated patient.

Clinical application of KI67: -

The Ki67 index serves as a reliable indicator of proliferative activity in mammary gland cancer. High Ki67 expression independently predicts cancer progression and prognosis. Immunostaining for Ki67 is commonly used, with a cutoff level of 10–14% positive staining indicating high-risk prognosis.Ki67 is highly expressed in malignant cells but rarely detected in normal cells.

Colorectal cancer: -

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Several methods have been used to study CTCs in colorectal cancer, depending on the physical characteristics of the cells or the expression of certain markers. Clinically speaking, CTCs have been investigated as biomarkers for prognosis, drug resistance monitoring, auxiliary staging of colorectal cancer, screening, and medication guidance. They have also been investigated as minimal residual disease (MRD) monitoring tools. It has been discovered that colon cancer can be identified by the circulation of CTC clusters.One potential early opportunity to predict target therapy response could be to evaluate the EGFR status in CTCs.

KRAS; (Kirsten rat sarcoma virus) is a gene that provides instructions for making a protein called K-Ras *KRAS* gene can also be amplified in colorectal cancer and tumors harboring this genetic lesion are not responsive to EGFR inhibitors.

Clinical application of KRAS: -

Patients with CRC harboring KRAS mutations tend to have a dismal prognosis. These mutations result in constitutive activation of the KRAS protein, persistently stimulating downstream signaling pathways related to cell proliferation and survival, ultimately leading to tumorigenesis. Specifically, patients with KRAS-mutant CRC face poorer outcomes, especially in the metastatic setting. KRAS mutation testing is now a routine clinical practice before treating metastatic CRC cases. Detect KRAS mutations exhibit favorable sensitivity and accuracy. For patients with stage IV metastatic CRC, KRAS mutation testing is essential.

In the identification and interpretation of ctDNA in colorectal cancer, epigenetic **markers (CpG island methylation)** are significant. A subset of colorectal cancers known as CpG island methylator phenotype (CIMP) are caused by an epigenetic instability pathway and are distinguished by extensive promoter CpG island site hypermethylation, which inactivates a number of tumor suppressor genes or other tumor-related genes.

Clinical application of CIMP: -

CIMP refers to the simultaneous hypermethylation of multiple genes' promoter-associated CpG-rich regions in CRC tissues. One of the most extensively researched gene promoters in CRC was **SEPT9** (This gene is a member of the septins family involved in cytokinesis and cell cycle control. This gene is a candidate for the ovarian tumor suppressor gene.).

Melanoma: -

After the existence of circulating melanoma cells was reported, numerous investigations employing various detection techniques were carried out, yielding inconsistent findings. The primary problem is the lack of epithelial cell adhesion molecule (EpCAM) expression in melanoma cells,

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which is the traditional marker used in the majority of CTC isolation techniques (Gaiser *et al*., 2018).

In light of this, alternate strategies based on marker-independent (based on the large size or density of primary melanoma cells) or maker-dependent (based on melanoma-specific antigens like MART-1, MAGE-A3, PAX-3, GalNac-T, HMW-MAA, and CD146 (MelCAM) strategies have been proposed. The magnetic Cell Search® Circulating Melanoma Cell Kit, the dual-step protocol immune-magnetic sorting and subsequent dielectrophoretic DEPArray separation, microfluidic chips and biosensors, and a new in vivo photoacoustic flow cytometry platform called "Cytophone" are among the most cutting-edge cell capture technologies (Gaiser *et* al., 2018).

Clinical application of PAX-3:-

The clinical applications of PAX-3, a biomarker associated with melanoma. PAX-3 is expressed in melanoma cells at various stages of disease progression, including primary lesions, circulating melanoma cells, and metastatic lesions. Detection of PAX-3 after overall treatment has been associated with significant decreases in CTCs. Identifying patients at high risk of relapse and determining optimal treatment choices are crucial in melanoma management. Molecular biomarkers like PAX-3 hold promise for improving patient prognosis and guiding therapeutic decisions

Clinical application of CD146: -

CD146 serves as a membrane antigen suitable for identification and enrichment in melanoma liquid biopsy. Liquid biopsy is a non-invasive method to detect tumor-specific markers in blood or other bodily fluids, aiding in early diagnosis and monitoring

Clinical application of HMW-MAA (High Molecular Weight-Melanoma-Associated Antigen):-

Due to its expression in a large percentage of melanoma lesions and its restricted distribution in normal tissues, HMW-MAA has been used as an immunotherapy target for melanoma. Immunotherapeutic approaches aim to harness the immune system to recognize and attack melanoma cells expressing HMW-MAA.

Future Perspectives and Conclusions:

In general, liquid biopsy refers to the collection and examination of analytes from different biological fluids (mainly blood, but occasionally urine, CSF fluid, or other secretions as well) that can be obtained using minimally invasive or non-invasive techniques. Liquid biopsy has become a powerful, non-invasive technique in the last ten years that can reveal crucial details about the molecular characteristics of solid tumors. Indeed, circulating tumor cells are demonstrating their potential in enabling real-time tumor staging, cancer monitoring, and the detection of therapy sensitivity and/or resistance. As previously mentioned, liquid biopsy has proven effective in treating

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patients with melanoma, breast, colorectal, and lung cancer to date. Therefore, it makes sense to assume that liquid biopsy-based evaluations will be used even more in routine settings once methodological procedures are harmonized and standardized across laboratories.

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