

# Liquid Biopsy

Subjects: **Oncology**

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Liquid Biopsy (LB) is a novel method for cancer diagnosis performed by analyzing and sampling of non-solid biological tissues, obtained primarily from blood, but also from other body fluids such as urine, saliva and cerebrospinal fluid.

pancreatic ductal adenocarcinoma

liquid biopsy

ctDNA

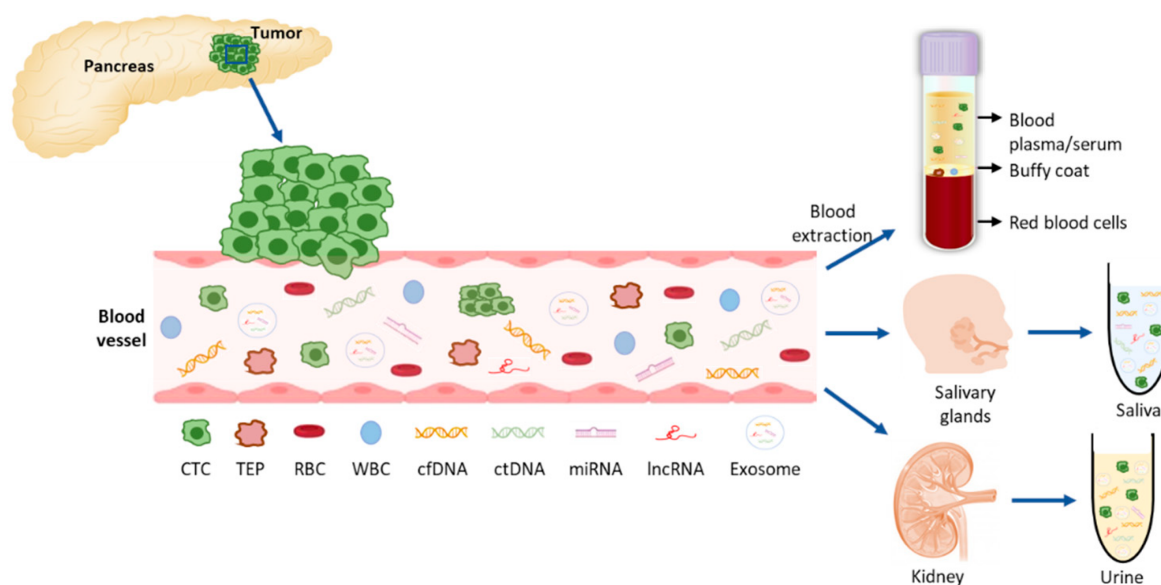
exosomes

CTCs

miRNAs

## 1. Introduction

Tumors and their metastases release biomarkers, mainly CTCs, cell free nucleic acids (cfDNA and cfRNAs), extracellular vesicles such as exosomes, and tumor educated platelets (TEPs), that can distantly reflect the disease (Figure 1). Therefore, liquid biopsies (LBs) represent a minimally invasive technique and allow diagnosis, real-time monitoring of cancer evolution and molecular follow-up of patients <sup>[1][2]</sup>. Also, LBs give us a better picture of the tumor heterogeneity than a tissue biopsy which only captures a specific area, since the whole tumor mass releases material into the blood <sup>[3]</sup>.



**Figure 1.** Liquid biopsy components in PDAC. Tumor cells (CTCs) are shedded from the tumor into the blood vessels where they can release their components: nucleic acids and exosomes with tumor-specific cargo material. For the analysis of these molecules, blood can be extracted and plasma or serum further processed for the extraction of the desired components. From the blood circulation, these molecules can be filtered to saliva and urine which can also be collected and further analyzed. CTC: circulating tumor cell; TEP: tumor educated platelet,

RBC: red blood cell; WBC: white blood cell; cfDNA: cell-free DNA; ctDNA: circulating tumor DNA; miRNA: micro RNA; lncRNA: long non-coding RNA.

Recent technological and molecular advances have increased our ability to detect and analyze LB components. In the following lines we will briefly introduce the different methods currently available for blood-based LBs (Table 1).

**Table 1.** Methods for isolation and analysis of liquid biopsy components in pancreatic cancer. Overview of the advantages and disadvantages of the described methods.

LB Component	Technique	Advantages	Disadvantages	Ref.
cfDNA	qPCR	Fast & Inexpensive High specificity	Lower sensitivity (0.1%) Detects only point mutations	[4][5][6][7][8][9][10]
	dPCR* (ddPCR, BEAMING)	High sensitivity (0.01%) & specificity	Detects only point mutations Expensive	
	NGS	High DNA input permits high throughput analysis and screen for unknown variants (WGS & WES) Can identify structural variants and copy number variations	Variable sensitivity (0.1% aprox.) Expensive	
Exosomes	Density-based isolation* (centrifugation)	Inexpensive Independent of marker expression	Time consuming High volume sample required Can damage exosomes Contaminated sample	[11][12][13][14][15][16]
	Size-based isolation	Fast & Inexpensive Independent of marker expression	Contaminated sample	
	Affinity-based isolation	High purity and specificity	Low sample yield	
	Commercial kits	Fast & Simple	Expensive	
CTCs	Immunoaffinity enrichment*	Positive enrichment: - Very specific - High capture efficiency & purity Negative enrichment: - Label-free CTCs obtained	Only one subpopulation captured  Lower purity	[11][4][17][18][19][20][21][22][23][24][25][26][27][28][29][30][31]

LB Component	Technique	Advantages	Disadvantages	Ref.
	Physical methods (size & density)	Represent tumor heterogeneity Fast & Simple Less expensive Label-free CTCs obtained	Must be followed with immuno-labelling techniques to distinguish CTCs	
cfRNAs	RT-qPCR	Fast & Inexpensive High specificity	Low sensitivity in samples with low abundance cfRNA	[32]
	ddPCR*	Higher sensitivity & accuracy Lower sample volume required More reproducible than qPCR	Tedious assay optimization	

LB: liquid biopsy; qPCR: real-time quantitative-PCR; dPCR: digital-PCR; ddPCR: droplet-dPCR, NGS: next generation sequencing; RT-qPCR: quantitative reverse transcription PCR; WGS: whole genome sequencing; WES: whole exome sequencing. \* Most used/gold standard.

## 2. Liquid Biopsy in Other Body Fluids for the Early Detection of Pancreatic Ductal Adenocarcinoma (PDAC)

Molecular analyses for the early detection of PDAC via LB are also being developed in other body fluids such as pancreatic juice, saliva and urine. The collection of pancreatic juice from the duodenal lumen is less invasive than other tissue biopsy collection methods, but it is still a relative invasive and difficult technique that needs to be performed by specialized personnel. Nevertheless, a number of molecular studies have been performed for the diagnosis of PDAC using pancreatic juice, mainly for the detection of *KRAS* mutations [33]. A meta-analysis of 16 studies that analyzed the diagnostic value of *KRAS* mutations revealed that the sensitivity and specificity levels for the diagnosis of PDAC were 0.59 and 0.87, respectively [34], and another meta-analysis of 39 studies assessing the diagnostic value of the four major altered genes in PDAC (*KRAS*, *CDKN2A*, *TP53* and *SMAD4*), telomerase activity, and combination assay revealed that the most reliable marker was telomerase activity with a sensitivity and specificity of 0.82 and 0.96, respectively [35]. However, these results should be analyzed with caution since they cannot distinguish early PDAC from intraductal papillary mucinous neoplasm (IPMN), or pancreas with low-grade PanIN, since alterations of *KRAS* and telomerase activity are also found in these lesions [33].

Saliva is a very convenient fluid for LB determinations since it can be easily and noninvasively obtained from patients, and it has been reported that it contains almost the same molecules as serum because of the high blood flow in salivary glands [5]. Molecular studies have been performed in saliva for the diagnosis of PDAC. Exosomes have been found in saliva in preclinical models and have been reported to discriminate PDAC, hence, they might be potential biomarkers for detecting PDAC [36]. A salivary transcriptomic analysis has identified a four-messenger

RNA panel (*MBD3L2*, *KRAS*, *ACRV1* and *DPM1*) that discriminates patients with PDAC from healthy controls with 0.9 sensitivity and 0.95 specificity [37]. MiRNAs miR-3679-5p and miR-940 have been reported to be down- and up-regulated in PDAC compared to healthy controls and benign lesions. The combined analysis of these miRNAs showed 0.7 sensitivity and 0.7 specificity in PDAC vs. noncancer [38]. The same group evaluated the expression of salivary lncRNAs and identified up-regulated levels of HOTAIR and PV1T in PDAC patients in comparison to healthy controls, with a combined sensitivity and specificity of 0.78 and 0.91 respectively. These values raised to 0.82 sensitivity and 0.95 specificity when differentiating PDAC from benign tumors [39].

Urine can be viewed as an ultrafiltrate of plasma and therefore may contain biomarkers that could assist with PDAC diagnosis [5]. Urine LB has the main advantages of allowing a completely non-invasive sampling and high volume collection, and has a lower proteome content than blood to avoid contamination of possible biomarkers [40]. Because of this, many metabolomic [41][42][43][44] and proteomic [45][46][47][48] studies have been conducted in order to identify possible biomarkers that can aid in the early identification of PDAC. With this purpose, Debernardi et al. have reported a urinary biomarker panel comprising LYVE1, REG1B, and TFF1 and PancRISK score that can discriminate patients with early stages of PDAC from control individuals and patients with benign hepatobiliary diseases [49].

Regarding cell free nucleic acids, detection of *KRAS* mutations in urine from PDAC patients has also been reported, and the detection rate and sensitivity are comparable to plasma LB [50]. Urinary miRNA biomarkers have also been analyzed and significant over-expression of miRNAs in PDAC Stage I versus healthy individuals (miR-143, miR-223, miR-30e) and Stage I versus Stages II-IV PDAC (miR-204, miR-143, miR-223) have been described [51]. A recent study also showed that the miR-3940-5p/miR-8069 ratio in urine exosomes is elevated in PDAC patients, suggesting that it may be a potential diagnostic tool for PDAC, especially in combination with CA19.9 [52].

### 3. Conclusions

Although there were many expectations set on the use of LB for early diagnosis, the truth is that the relatively low sensitivity and specificity of current techniques does not allow its use for these purposes. In addition, the available studies suggest that patients with PDAC in whom ctDNA is detected at the time of diagnosis have a poor prognosis and have a high chance of relapse after surgery, so it is advisable to develop more sensitive techniques that allow diagnosing tumors in earlier stages and provide patients with a better prognosis. It is possible that, with the development of ultrasensitive techniques, the joint use of different biomarkers and epigenetic marks, the sensitivity of LB will increase without losing specificity, and LB could be applied in PDAC screening.

The different LB methods have been shown to be a reliable biomarker in relation to the prognosis of patients with PDAC for both PFS and OS. Furthermore, its variations throughout treatment predict response or resistance to treatment several weeks in advance, so it could be used to guide treatment based on the evolution of the biomarker. In addition, the study of the characteristics of CTCs, ctDNA, exoDNA and miRNA can help us to better characterize the tumor and to identify potential therapeutic targets that facilitate the selection of treatment.

However, it is necessary to standardize and validate the methodology to be used in the different LB modalities. In addition, the usefulness of other LB modalities should be explored, such as lncRNAs or TEPs. On the other hand, the usefulness of less invasive sources of ctDNA such as urine or saliva needs to be further investigated.

Finally, it should be remembered that in order to apply LB to clinical practice, it is necessary to reduce costs, standardize protocols, and have data generated in the context of large-scale prospective clinical trials that confirm that the information provided contributes significantly to improving therapeutic results in PDAC patients.

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