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Advances in Liquid Biopsy for Early Detection and Monitoring of Pancreatic Cancer

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ABSTRACT

Pancreatic cancer is a rather aggressive form of malignancy. It characteristically presents at an advanced stage and progresses aggressively. The liquid biopsy has revolutionized the diagnosis and monitoring approach. The present review provides details on current progress, clinical utility, and the challenge in applying the method for managing pancreatic cancer. These can shed light on tumor biology, molecular changes, and treatment responses with the examination of tumor-derived fluids like ctDNA, CTCs, and exosomes. Among the technologies which could enhance sensitivity and specificity to make this a more viable test for early detection and real-time monitoring are next-generation sequencing and microfluidics and other highly advanced exosome isolation techniques. Such clinical studies have only revealed recent times that liquid biopsy is indeed useful in the detection of hallmark mutations, such as KRAS and TP53, prognosis assessment, and therapeutic efficacy monitoring. Recent trends in multi-omics integration and artificial intelligence-driven biomarker discovery as well as liquid biopsy-based developing point-of-care diagnostic devices highlight the potential of revolutionizing personalized medicine. Despite its promise, some challenges that include low abundance of biomarkers, lack of standardization, and high costs characterize what limits acceptance of liquid biopsy in clinical practice. This review outlines the role which liquid biopsy can play in conquest of the challenges of diagnosis and therapy of pancreatic cancer. Concomitantly, it discusses future requirements for research, technological breakthroughs, and a collaborative attitude toward overcoming prevalent limitations. Multidisciplinary approaches in the fields of multi-omics, artificial intelligence, and scalable diagnostic tools hold the promise of enhanced early detection of pancreatic cancer and optimization of treatment strategies in the patients themselves.

INTRODUCTION

Pancreatic cancer is still considered one of the deadliest malignancies in the world, and the share of mortality due to deaths from cancers that it holds is significant. Its bleak outlook is basically the result of an asymptomatic progressive course and very late detection. With an estimated five-year survival rate of less than 10%, pancreatic cancer is the seventh leading cause of death from cancer internationally (Clancy, 2023; Rahib et al., 2014). This malignancy is very aggressive, with high metastatic tendency and also resistant to conventional therapies. The late-stage diagnosis of pancreatic cancer is a major clinical challenge because this disease often is asymptomatic in its early stages or manifests nonspecific symptoms such as abdominal pain, weight loss, and jaundice, which usually start occurring when the disease is already advanced (Ryan et al., 2014; McGuigan et al., 2018). These are the factors that make early detection an essential yet unmet need in improving the survival rates of patients.

Early detection and monitoring of pancreatic cancer improve clinical outcomes. In studies, it has been shown that if pancreatic cancer is detected at an early stage with a localized tumor that is resectable, the five-year survival

rate almost be 37% (Vincent et al., 2011; Howlader et al., 2021). This is especially relevant because surgical resection, though not generally used as a first-line treatment for this disease, remains the only curative treatment available for pancreatic cancer. The diagnostic techniques used today, such as imaging techniques, like CT, MRI, and EUS, and tissue biopsies, are all deficient in one or more ways. Some are invasive and expensive, and others lack sensitivity to detect small, early-stage tumors (Canto et al., 2013; Sausen et al., 2015). Therefore, there is a pressing need for highly sensitive and non-invasive diagnostic tools in order to achieve early detection, monitoring of the progression of the disease, and the response of the treatment.

Liquid biopsy is one of the promising non-invasive diagnostic modalities in cancer, offering new means of detection and monitoring of cancer. Liquid biopsy is defined as the assessment of tumor-derived components present in body fluids, such as blood, urine, and saliva, which is different from the traditional tissue biopsy. The components include circulating tumor cells (CTCs), cell-free DNA (cfDNA), circulating tumor DNA (ctDNA), circulating RNA, including microRNAs and long non-coding RNAs, exosomes, and tumor-derived proteins (Wan et al., 2017; Bardelli & Pantel, 2017). Liquid biopsy enables the real-time assessment of molecular features of a tumor and thus reveals much about genetic and epigenetic profiling of a tumor involved (Hrstka et al., 2016). This approach is very useful for pancreatic cancer as it avoids the disadvantages of tissue biopsies that include sampling bias, invasiveness, and inability to capture heterogeneity of the tumor (Makohon-Moore & Iacobuzio-Donahue, 2016).

Liquid biopsy for pancreatic cancer has a very wide and diverse range of clinical applications-from early detection, towards prognosis and even follow-up in monitoring the treatment response and disease recurrence. For example, ctDNA can be exploited in the screening of specific mutations like KRAS and TP53 that typically manifest in pancreatic cancer and thus become a potential biomarker in the early screening of the disease (Kamisawa et al., 2016; Zill et al., 2015). CTCs and exosomes have also been explored as markers of tumour burden and metastatic potential and, thus, for prognosis and treatment (Castellanos-Rizaldos et al., 2018; Melo et al., 2015). Liquid biopsy also enables the dynamic surveillance of progression and assessment of disease response during real-time therapy, thereby enabling more personalized treatment of cancer (Siravegna et al., 2017).

But translating this to clinical applications is not without challenges. Some of the problems associated with this test include low circulating biomarkers of tumor origin, lack of standardization of sample acquisition and processing protocols, and higher cost of using high technology Yachida et al., 2016; Bettegowda et al., 2014). However, with the developments of next-generation sequencing (NGS) and bioinformatics technologies, the limitations are gradually being overcome; thus, liquid biopsy can be soon implemented in daily clinical practice settings (Heitzer et al., 2015; Alix-Panabières & Pantel, 2021).

Liquid Biopsy Overview

Liquid biopsy is the diagnostic approach that examines tumor-derived materials in bodily fluids, including blood, urine, cerebrospinal fluid, and saliva. This technique is unique because it does not require the invasive surgical procedures used to obtain tumor samples in conventional tissue biopsies, allowing the detection and monitoring of cancer through simple blood draws or other non-invasive collection methods. Liquid biopsy depends on the identification and characterization of the tumor-associated biomarkers of circulating tumor cells, cell-free DNA, and other tumor materials circulating in the blood or in the bodily fluids from which it could be expelled by a developing tumor (Wan et al., 2017). The technique has received much attention in the field of cancer diagnosis on account of the possibility of providing real-time insight into the molecular landscape of a tumor, which hence enable early detection, personalize treatment, and allow continuous monitoring of the efficacy of therapy.

Liquid biopsy is indeed more particularly relevant in the context of cancers such as pancreatic cancer, as those biopsies require tissue that is not possible to be accessed due to the deep anatomical location of the tumor. Moreover, it overcomes the major limitations of conventional methods by providing a non-invasive, repeatable, and dynamic method for tumor profiling (Bettegowda et al., 2014). It captures the molecular heterogeneity of tumors and allow for the detection of minimal residual disease, thus becoming a very useful tool in modern oncology.

Types of Analytes in Liquid Biopsy

Circulating Tumor Cells (CTCs)

CTCs are cancer cells breaking off from the primary tumor and entering the bloodstream. They are significant in metastasis and can be useful biomarkers in cancer detection, prognosis, and treatment monitoring. CTCs can be isolated and characterized through immunomagnetic separation, microfluidics, and size-based filtration (Alix-Panabières & Pantel, 2021). The presence of CTCs in pancreatic cancer has been associated with disease progression and poor prognosis. However, their low abundance in circulation poses a challenge to their routine clinical use (Visser et al., 2018).

Cell-Free DNA (cfDNA) and Circulating Tumor DNA (ctDNA)

cfDNA is pieces of DNA that are released into the bloodstream during apoptosis and necrosis processes. Of

all cfDNA, the subfraction known as ctDNA is distinct because it originates directly from cancer cells and carries cancer-specific alterations such as mutations, copy number alterations, and DNA methylation profiles (Wan et al., 2017). For example, in pancreatic cancers, the analysis of ctDNA have identified actionable mutations such as KRAS mutations and TP53 mutations that might justify an earlier diagnosis and subsequent treatments tailored for them (Mouliere et al., 2018). Techniques such as digital PCR and NGS improved the sensitivity and specificity in the detection of ctDNA.

Extracellular Vehicles (EVs) and Exosomes

Exosomes included, extracellular vesicles are nanosized particles released by cells into the extracellular environment. They contain various biomolecules: proteins, lipids, DNA, and RNA, which constitute the molecular characteristics of the parent cells (Melo et al., 2015). Exosomes obtained from pancreatic cancer cells contain diagnostic markers like glypican-1 (GPC1) that, to a significant degree, differentiated pancreatic cancer patients from healthy controls (Kahlert et al., 2014). Exosomes have been implicated in cell-to-cell communication and cancer progression, thereby serving as an exciting target for therapy.

Blood RNA (MicroRNAs, Long Non-Coding RNAs, etc.)

The cancer cells are believed to release into circulation RNA molecules containing miRNAs and lncRNAs. These RNA species released into circulation remain stable, mainly due to encapsulation by EVs or association with protein complexes (Mitchell et al., 2008). Some specific miRNAs have been indicated, such as miR-21 and miR-155, to serve as potential biomarkers for the detection of pancreatic cancer and thus useful for early detection and prognosis (Wang et al., 2015). Recently, lncRNAs also proved to be new diagnostic and prognostic markers.

Circulating Proteins and Metabolites

Besides cytokines, growth factors, and metabolites, circulating proteins and metabolites are biomarkers in liquid biopsy. For example, carbohydrate antigen 19-9 (CA19-9) is one of the most recognized circulating protein biomarkers for pancreatic cancer. Although not specific for pancreatic cancer, levels correlate with disease burden and treatment response, making it potentially a useful adjunct to other diagnostic approaches (Humphris et al., 2012). Recent advances in proteomics and metabolomics are opening up the potential of circulating biomarkers for the detection and monitoring of cancer.

Advantages over Traditional Tissue Biopsies

Non-Invasive

Liquid biopsy avoids surgical processes that may result in patient suffering and dangers during tissue biopsy. It risks blood flow and infections. Such techniques are useful especially to pancreatic cancer patients, given that obtaining tissue samples may be quite hard since the anatomy of the area of interest presents a barrier, making sampling and even procedures hazardous (Hrstka et al., 2016).

Real-Time Monitoring

Unlike tissue biopsy, which provides a static view at one point in time, liquid biopsy allows for dynamic monitoring of the tumor evolutionary process and its response to treatment. This method helps clinicians detect emerging resistance mutations and change their strategies for treatment accordingly (Siravegna et al., 2017). Liquid biopsy requires only easy draws of blood or less-invasive methods of sample collection that minimize procedural risks and allows repetition multiple times-making it convenient for long-term monitoring of a disease (Wan et al., 2017).

Molecular Alterations in Pancreatic Cancer

Pancreatic cancer, largely pancreatic ductal adenocarcinoma, is an aggressively malignant neoplastic disease defined by the collection of genetic and epigenetic alterations that mediate tumor initiation, progression, and metastasis. These are crucial in the generation of the disease and have deep implications for its early detection, prognosis, and therapy. The liquid biopsy now emerges as a very transformative tool, capable of collecting these alterations non-invasively, therefore allowing for monitoring of pancreatic cancer in real-time.

Common Genetic Mutations and Biomarkers of Pancreatic Cancer

- KRAS Mutation In the context of pancreatic cancer, the most commonly mutated gene is KRAS oncogene, in which mutations are identified in more than 90% of cases (Kanda et al., 2012; Ryan et al., 2014). KRAS mutations most commonly occur at codons 12, 13, and 61. Tumor cells grow more, are more invasive, and more resistant to apoptosis through activation of the Ras protein with hyperactivation of downstream MAPK and PI3K-AKT pathways (Hidalgo, 2010; Hezel et al., 2006). It has been particularly specific for PDAC and was the only one that many scientists studied pertaining to a diagnostic and prognostic biomarker in analysis in ctDNA of liquid biopsy (Mouliere et al., 2018).
- TP53 Mutation Mutations in TP53, a key tumor suppressor gene, are observed in approximately 50–75% of pancreatic cancers (Zhou et al., 2018). These mutations result in the loss of p53 function, which normally regulates DNA repair, apoptosis, and cell cycle control. Consequently, TP53 mutations promote genomic instability and tumor progression. Liquid biopsy can detect TP53

mutations in ctDNA, providing insights into tumor dynamics and patient prognosis (Bettegowda et al., 2014).

- CDKN2A Inactivation Almost 90% of pancreatic cancer samples harbor mutations of the CDKN2A gene encoding for the tumor suppressor p16 (Hezel et al., 2006). The action of p16 helps regulate the transition from the cell cycle's G1-S phase by inhibiting the activities of cyclin-dependent kinases CDK4/6. The lack of p16 leads to uncontrolled proliferation in cells. Detection of alterations in CDKN2A in ctDNA and exosomal DNA might open new possibilities for its application as a marker for early pancreatic cancer liquid biopsy (Zill et al., 2015).
- SMAD4 Mutation SMAD4 is an intracellular mediator of the TGF-β signaling pathway and is inactivated in about 55% of pancreatic cancers (Hingorani et al., 2003). The mutation tends to be seen in more advanced disease, metastatic disease, and poor prognosis. Alterations in SMAD4 are detectable in ctDNA, offering the possibility for patient stratification based on disease severity and the development of future therapies (Makohon-Moore & Iacobuzio-Donahue, 2016).
- Other Key Mutations Other genes commonly mutated in pancreatic cancer include the alterations to the ARID1A, BRCA1/2, and RNF43 genes (Waddell et al., 2015). For example, mutations in the BRCA1/2 gene typically signify that there is an inability to properly repair DNA and therefore are susceptible to PARP inhibitors, thus making it a target for therapy. These mutations can also be identified in liquid biopsies to aid in therapies (Golan et al., 2019).

How Liquid Biopsy Captures These Alterations for Diagnostic Purposes

Liquid biopsy is a non-invasive platform that is used to detect and monitor this sort of genetic alteration, providing real-time insights into tumor biology:

1. Circulating Tumor DNA (ctDNA) ctDNA is a broken, tumor-derived piece of cell-free DNA, bearing the genetic mutations of the cancer. Highly sensitive technologies in the form of ddPCR and NGS help to identify these mutations in KRAS, TP53, CDKN2A, and SMAD4 in circulating cell-free DNA (ctDNA). The diagnostic yield of analyses from ctDNA is promising with encouraging results obtained in the search for early pancreas cancer and monitoring progressions of a disease (Bettegowda et al., 2014; Zill et al., 2015).
2. Circulating Tumor Cells Circulating Tumor Cells offer an all-encompassing analysis of the cancer that is both genomically and transcriptomically rich. These can be isolated for the purpose of testing mutations such as KRAS and TP53, and analyzing tumor heterogeneity. Microfluidic platforms and separation by immunomagnetic capabilities have improved significantly the efficiency for isolating these cells in a patient with pancreatic cancer (Visser et al., 2018).
3. Exosomal DNA and RNA Tumor cells in every stage of progression release exosomes, which are nanosized extracellular vesicles, harboring DNA, RNA, and proteins that characterize the molecular signature of the tumor. For example, mutations in the KRAS gene were found in the DNA carried within exosomes. Circulating miRNAs carried within exosomes, for example, miR-21 and miR-155, were established to have diagnostic value in pancreatic cancer (Melo et al., 2015; Wang et al., 2015). Exosomes that are positive for Glypican-1 (GPC1) are another potential biomarker with much specificity to the diagnosis of pancreatic cancer (Kahlert et al., 2014).

Molecular Markers in Liquid Biopsy

- DNA methylation is disrupted in pancreatic cancer. There is the hypermethylation of CDKN2A, a tumor suppressor gene, and hypomethylation of oncogenes in ctDNA. These epigenetic changes are being evaluated as markers for the early diagnosis and prognosis of patients (Rah et al., 2021).
- Tumor Mutational Burden (TMB) Liquid biopsy: It assesses the tumoral mutational burden through the evaluation of ctDNA. High TMB has been found to be correlated with better responses to immune checkpoint inhibitors, and hence can be a biomarker for immunotherapy in pancreatic cancer patients. Chowell et al., 2018.
- Circulating miRNAs like miR-21, miR-155 and miR-196a are recognized as a group of potential non-invasive biomarkers of pancreatic cancer. It controls proliferation, apoptosis and chemoresistance processes. Being very stable in circulation, liquid biopsy applications hold enormous potential for its detection (Wang et al., 2015).
- BRCA1/2 and HRD Status Liquid biopsy can detect homologous recombination deficiency (HRD) due to BRCA1/2 mutations. This biomarker is important for the selection of patients who could be treated with PARP inhibitors, a targeted therapy for pancreatic cancer (Golan et al., 2019).
- Metabolic biomarkers with the advancement of metabolomics, certain specific alterations in the metabolism of amino acids and lipid pathways have been identified in pancreatic cancers. Such alterations are detectable in blood and may thus

potentially have potential as early clinical markers (Mayers et al., 2014).

Progressive Improvement in Liquid Biopsy in the Pancreas Cancer

Liquid biopsy has presented as a very recent innovation on the horizon for the oncologist to use with its diagnostic monitoring of cancer-type biomarkers by circumventing the classical sampling process directly by an invasive biopsy. In fact, it should be applied, given the severe nature of its manifestation and by extension the restricted sensitivity of such age-old detection, to diagnose or monitor pancreatic ductal adenocarcinoma, referred to as PDAC. Liquid biopsy facilitates early detection of presence, prognosis, and real-time monitoring of responses to treatment with tumor-derived components, including ctDNA, CTCs, and exosomes. The applications and developments in technologies and clinical application have enormously upgraded the usage of liquid biopsy for the management of pancreatic cancer.

Technological Advancement

Next-generation sequencing (NGS) has transformed the analysis of ctDNA, a tumor-derived subset of cell-free DNA (cfDNA) released into the bloodstream during apoptosis or necrosis. NGS allows the profiling of genetic alterations, including point mutations, structural variations, and methylation changes, with high sensitivity and specificity. With molecular barcoding, error-correction strategies, and ultra-deep sequencing, low frequency mutations in ctDNA can now be identified in the early stages of pancreatic cancer. It is now very likely to be detected with relatively good accuracy for identification of mutation in genes that often get mutated in PDAC, such as KRAS, TP53, CDKN2A, and SMAD4, as mentioned in Mouliere et al., 2018; Wan et al., 2017. All these developments permit identification of actionable mutations at a stage when it can be beneficial in the clinical sense and may be used for possibility of treatment options in terms of personalizing medicine.

Isolation and characterization of CTCs have also become possible through the advancement of technology. Enumeration of rare cancer cells emanating from the parent tumor into the bloodstream - CTCs, has been one of the most fundamental biomarkers of assessing heterogeneity of the tumor and metastatic potential. Microfluidic platforms such as the CTC-iChip are designed for enriching and isolating CTCs based on size, deformability, and surface markers from blood samples (Visser et al., 2018). Single EpCAM-based immunomagnetic enrichment techniques have improved sensitivity to detect CTCs to considerable extents, but there are still major challenges in identifying mesenchymal-like CTCs that may not express EpCAM. However, breakthroughs in recent single-cell analysis, such as through RNA sequencing, have now enabled one to study single CTCs and thus initiate the unveiling of insights into tumor heterogeneity and mechanisms of drug resistance.

These nanoscale extracellular vesicles secreted by the tumor cells carry promising analytes in liquid biopsy. Exosomes carry tumor-specific DNA, RNA, proteins, and lipids; hence, improved isolation techniques in the form of ultracentrifugation, immunoaffinity-based methods, and microfluidics have elevated the yield and purity of the exosome sample. Advanced tools in the form of nanoparticle tracking analysis and nanoscale flow cytometry can do an elaborative characterization of the exosomal cargo. Exosomes carrying GPC1 are a very high specificity marker between pancreatic cancer patients and healthy people and, as such, diagnostic potential (Melo et al., 2015; Kahlert et al., 2014).

Diagnostic Uses

The main difficulty in the treatment of pancreatic cancer is that this disease is normally diagnosed at its late stages. The nonspecific symptoms associated with the disease arise only when it has progressed significantly. Liquid biopsy overcomes this drawback by detecting, at an early stage, tumor-specific biomarkers from bodily fluids. Circulating tumor DNA is a valid marker for diagnosis. According to a study on ctDNA, it has been said that mutations in KRAS-the hallmark mutation related to pancreatic cancers-can be identified even in its initial stages. In several analyses, mutations for KRAS can be found to be present in 90% or more of patients suffering from pancreatic cancers, and thus, this test is an extremely sensitive method of diagnosis (Zill et al., 2015). Other circulating microRNAs, including miR-21 and miR-155, also have the potential to be very valuable in early biomarkers due to the stability of these microRNAs in circulation, and high correlation with tumor progression (Wang et al., 2015).

Liquid biopsy technologies advanced with increased sensitivity and specificity due to better detection technologies, such as digital PCR and NGS. GPC1-positive exosomes have near 100% specificity in differentiating between patients suffering from pancreatic cancer and healthy subjects (Melo et al., 2015). The sensitivity and specificity of the diagnosis may increase with the help of various biomarkers, including ctDNA, exosomal markers, and circulating proteins that provide a comprehensive molecular signature of the tumor.

Prognostic Applications

It has been particularly helpful also for prognostic purposes and the follow-up course of the disease. The extent to which it agrees with tumor load and metastatic potential can be estimated, and such information provides the basis for judgment on the prognosis. Advanced disease usually goes along with grave

survival, which corresponds with higher levels of ctDNA. Dynamic changes in ctDNA profiles over the course of disease can provide real-time information about the dynamics of tumor evolution and the emergence of resistance to therapy (Bettegowda et al., 2014).

The second important prognostic agent is circulating tumor cells. A high number of CTCs has been linked to shorter overall survival and increased metastatic potential in pancreatic cancer patients. Genomic and transcriptomic single-cell analysis of CTCs has facilitated the ability to indicate changes associated with metastasis and drug resistance, thus rationalizing the role of CTCs in helping to decide clinical decisions (Visser et al., 2018). Liquid biopsy allows for a more dynamic and individualized assessment of prognosis for the patient by embracing the heterogeneity of the tumor.

Monitor Treatment Response

Probably the most exciting aspects of liquid biopsy include the possibilities for tracking responses to therapy in real time and identification of early recurrence of the disease. ctDNA is considered a dynamic biomarker of the tumor burden and response to treatment. Levels of ctDNA during chemotherapy or targeted therapy that are normally decreasing indicate a response to treatment. However, stable or increasing levels of ctDNA may indicate resistance or disease progression (Zill et al., 2015). This test's greatest utility lies in identifying minimal residual disease and very early recurrence. Traditional imaging catch only a disease that is macroscopic, that is to say too late. In this context, addition of ctDNA and exosomal markers offer the possibility of detection of tumor re-growth on molecular basis thus treatment decisions at proper times (Wan et al., 2017). Serial sampling through liquid biopsy offers a perspective of longitudinal dynamics of tumors which allows tailoring treatment so that long-term outcomes are enhanced.

Challenges in Liquid Biopsy for Pancreatic Cancer

Although liquid biopsy has very promising potential as a transformable tool for pancreatic cancer management, it is not without its challenges. The foregoing difficulties must be addressed to ensure general clinical acceptance and maximize its diagnostic and therapeutic potential. The main challenges include low concentration of tumor-derived biomarkers, lack of standardization in methodologies, variability in sensitivity and specificity, and high costs as well as limited accessibility of advanced technologies.

Low Circulating Biomarker Concentration

The other significant challenge that is inherent with the detection and isolation of biomarkers in liquid biopsy is that of their natural occurring low concentration in blood. The main candidates circulating include ctDNA, CTCs, and exosomes. Pancreatic cancer, however, is primarily known to possess very poor vascularity of the tumor microenvironment compared to most cancers (Wan et al., 2017).

- ctDNA: Although nowadays a considered excellent biomarker for early detection and monitoring, its fraction of the total circulating cell-free DNA generally is less than 1% in early diseases, and detection by conventional methods is really hard (Mouliere et al., 2018). The detection of rare mutations of ctDNA, such as KRAS, poses technical challenges that are expensive, especially by using ultra-deep sequencing or droplet digital PCR.
- CTCs: Not very scarce and in very limited quantities per ml of blood. Lower count together with heterogeneity in their phenotypes, for instance epithelial as compared to mesenchymal-like CTCs makes it very much a challenge job of its reliable detection and isolation much difficult (Visser et al., 2018).
- Exosomes: Still much more abundant than both ctDNA and CTCs; yet the problem is that in a background mainly constituted by exosomes derived from non-tumorous cells only those exosomes tumor-specific are possible to purify. It means that all microfluidics and immunoaffinity technologies already under development cannot ensure such isolations to be purer and specifically enough (Melo et al., 2015).

Lack of Standardization in Sample Collection, Processing, and Analysis

Another significant challenge that liquid biopsy face in the context of pancreatic cancer is the standardization of methodologies used for sample collection, processing, and analysis. Variability between laboratories in standard operating procedures not allow reproducibility and comparability of results.

- Sample Collection: type of sample, plasma vs serum; type of tube; time to process: all factors are hugely influential to the quality and integrity of the biomarkers, particularly more so for ctDNA and exosomal RNA (Alix-Panabières & Pantel, 2021). The longer it takes to process, the further degraded the nucleic acids become or it simply allows background DNA leakage from lysed blood cells to interfere with subsequent analyses.
- Isolation Methods. The methods of isolation of ctDNA, CTCs, and exosomes differ in each. Some of them include centrifugation, microfluidics, and immunoaffinity capture. They differ on efficiency and purity, which might make it hard to standardize a threshold value that could serve as a universal way of quantifying a biomarker.
- Analytical Tools: The variables that can cause heterogeneity in interpreting results are

sequencing platforms, bioinformatics pipelines, and thresholds set for analytical analyses. Uniform sequencing depth, variant calling, and reporting guidelines could unify all factors irrespective of clinical application (Siravegna et al., 2017).

Heterogeneity in Sensitivity and Specificity of Current Methods

Good variations in sensitivity and specificity of the techniques assessing the liquid biopsy are reported despite the advancement in the detection technologies, primarily in the case of pancreatic cancer. This may influence the reliability of the results and limit clinical utility.

- Sensitivity Problems: Different factors determine the sensitivity of liquid biopsy technologies. They consist of the stage of tumor, the burden of the tumor and type of biomarker released. A tumour of an early stage pancreatic cancer sheds lesser biomarker. Henceforth, with increasing rate of sensitivity, the rate of the method is reduced. For instance, only exosomes containing GPC1 can be used in the diagnosis of pancreatic cancer; it is very specific but not sensitive in many cases (Melo et al., 2015).
- False Positives and Negatives: This could be explained by the presence of DNA or exosomes, not of tumour-derived source, false negatives of the biomarkers having very low abundance, and technical limitations that make it inapplicable to detect. Essentially, multi-marker approaches and higher order error correction schemes have to be taken in order to bring in further improvements to the assay in terms of its sensitivity (Zill et al., 2015).
- Tumor Heterogeneity: Pancreatic cancer is characterized with genetic and molecular heterogeneity that makes the results from liquid biopsy very challenging to interpret. A single biomarker cannot cover all the alterations that are present in the tumor; therefore, highly comprehensive profiling methods need to be applied.

Expensive Technologies Combined with Their Poor Availability

This further exacerbates the problem because liquid biopsy technologies are pricey, and access is severely limited and unacceptably for most scenarios in a world that remains largely resource-constrained.

- High Equipment and Consumable Costs: The technologies comprise NGS, ddPCR, and high-end microfluidic platforms, which are expensive equipment and reagents that most healthcare facilities cannot afford (Wan et al., 2017). For instance, ultra-deep sequencing of ctDNA has very high sequencing costs, which makes it not so feasible for routine clinical use.
- The complexity of methods in liquid biopsy mandates specialized expertise about sample processing and data analysis and interpretation. Shortages of trained staff in most of the regions prevent these techniques from scaling up even further.
- Limited Access: Further, liquid biopsy technologies are so advanced and can only be used in specialized centers found in high-income countries. On this basis, this limit health care institutions, especially those in low-and middle-income settings, where infrastructure and tools for applying such technologies are not well available, thus worsening disparities in the care of patients with cancer (Mouliere et al., 2018).

Liquid Biopsy Technologies in Clinical Trials and Studies

Liquid biopsy in the clinic of pancreatic cancer revealed that all problems found with utility of conventional techniques can be nicely overcome.

Detection of KRAS Mutations in ctDNA

KRAS mutations are the hallmark of pancreatic ductal adenocarcinoma and frequently detected in ctDNA. A study conducted by Zill et al. in 2015 used NGS to study ctDNA of pancreatic cancer patients and obtained a concordance rate above 90% between the ctDNA results and those obtained from tissue biopsy, indicating a high promise of using ctDNA as a diagnostic marker.

Glypican-1 (GPC1)-Positive Exosomes

Melo et al. (2015) have carried out a study, which shows the diagnostic potential of GPC1-positive exosomes in pancreatic cancer. The authors showed that GPC1-positive exosomes can distinguish between pancreatic cancer patients and healthy donors with nearly 100% specificity, making them a promising biomarker for early detection.

Prognostic Utility of CTCs

Enumeration of CTCs has been studied as a prognostic biomarker in pancreatic cancer. Visser et al. (2018) demonstrated that elevated counts of CTCs were associated with shorter overall survival, whereas the single-cell sequencing of CTCs revealed mutations associated with metastasis and drug resistance mechanisms.

Early Recurrence Detection based on ctDNA

Levels of ctDNA in patients subjected to surgical resection for pancreatic cancer were measured and followed during an investigation conducted by Cohen et al. (2017). Patients whose levels of ctDNA could still be detected after surgery faced more risk of occurring events; hence, ctDNA can be used as a suitable marker

for the measurement of MRD and very early recurrence monitoring.

Liquid Biopsy for the Surveillance of Neoadjuvant Therapy

Groot et al. (2021) investigated the use of ctDNA as a biomarker for monitoring the response to neoadjuvant therapy in patients with pancreatic cancer. The level of ctDNA was inversely proportional to the reduction in size observed in imaging studies; therefore, drug response may be monitored in real time.

Recent Applications

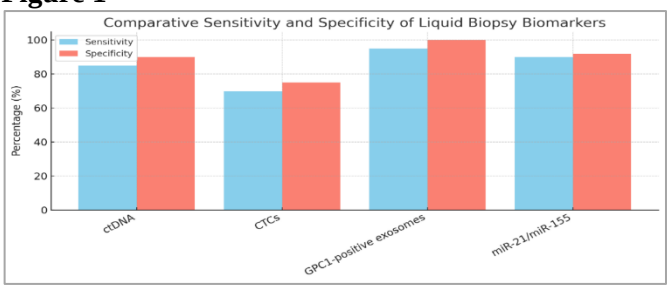
Early Detection

Early detection plays a crucial role in pancreatic cancer because it proceeds symptomatically with a poor prognosis. Liquid biopsy offers a less-invasive alternative over traditional tissue biopsies and other imaging methods.

ctDNA-based detection

ctDNA analysis has been very promising in identifying mutations in genes such as KRAS, TP53, and SMAD4, which are common in pancreatic cancer. In a multi-center study, Garlan et al. (2017) demonstrated that ctDNA could detect pancreatic cancer with a sensitivity of 73% and specificity of 98%, even in early-stage disease.

Figure 1



Exosomal Biomarkers

GPC1-positive exosomes have emerged as a highly specific biomarker for the detection of pancreatic cancer. Melo et al. (2015) reported that GPC1-positive exosomes were more sensitive than the traditional markers, such as CA19-9, particularly in early-stage patients.

MicroRNAs (miRNAs)

Exosome circulating miRNAs, such as miR-21 and miR-155, have been highly validated as early detection biomarkers. Wang et al. (2015) showed that these miRNAs are very sensitive and specific for the differentiation between pancreatic cancer patients and healthy individuals.

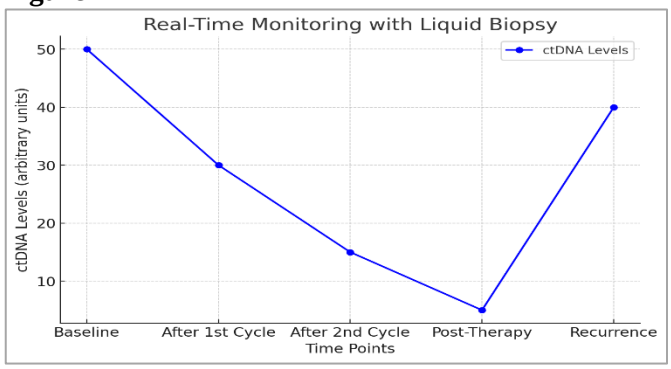
Treatment Monitoring

Liquid biopsy enable the real-time monitoring of treatment response and provide an essential window of information that might be critical for making clinical decisions.

Kinetics of ctDNA during Treatment

Concentration changes of ctDNA after treatment indicate the burden of the tumor and assess the response to the therapy. According to Ren et al. (2019), ctDNA levels monitored in patients treated with chemotherapy for advanced pancreatic cancer exhibited a decline where corresponding regressions in the tumors occurred and stabilization or an increase that shows resistance.

Figure 2



In a nutshell, Groot et al. (2021) demonstrated that integration of ctDNA monitoring with imaging enhances the evaluation of treatment response. More frequent pathologic complete response was observed among undetectable patients for levels of ctDNA after neoadjuvant therapy.

Detection of emerging resistance

Liquid biopsy identifies secondary KRAS mutations and other novel resistance mutations early, which may guide timely readjustment of treatment regimens. The technology is useful for patients who are on targeted therapies or immunotherapies (Siravegna et al., 2017).

Prognostication

Liquid biopsy offers prognostic value since this technology can capture tumor heterogeneity and predict clinical outcomes of the disease.

CTCs as Prognostic Biomarkers

High counts of CTCs also correlate well with a bad prognosis of pancreatic cancer. According to a prospective study, in the work conducted by Bidard et al. (2018), there was an observation wherein patients having fewer than 3 CTCs per 7.5 mL of blood have a significantly long survival compared with those patients having more counts of CTCs.

Risk Stratification Through Molecular Profiling

It allows the study of tumour evolution and the mechanism of resistance for guiding the risk stratification and treatment decisions for CTCs through single-cell sequencing (Visser et al., 2018).

ctDNA and Survival Prediction

The presence of ctDNA at the baseline and during the treatment has been well correlated with both progression-free and overall survival. Bettegowda et al. (2014) have found ctDNA to be detectable in as much as

88% of metastatic pancreatic cancer patients with higher levels predicting worse outcomes.

FDA-Approved and Emerging Liquid Biopsy Tests

Guardant360®

The FDA-approved Guardant360® assay is a ctDNA-based NGS test, offering comprehensive genomic profiling. It identifies actionable mutations in pancreatic cancer, such as KRAS and BRCA1/2, which help in providing personalized treatment.

FoundationOne Liquid CDx

FoundationOne Liquid CDx is another FDA-approved liquid ctDNA test that covers over 300 genes and offers information on TMB and MSI. It was used with immunotherapy and targeted therapy in pancreatic cancer.

Signatera™

Signatera™ is a personalized ctDNA test that is meant for follow up minimal residual disease and recurrence. It has proven to be useful for the early detection of recurrences in pancreatic cancer than with imaging.

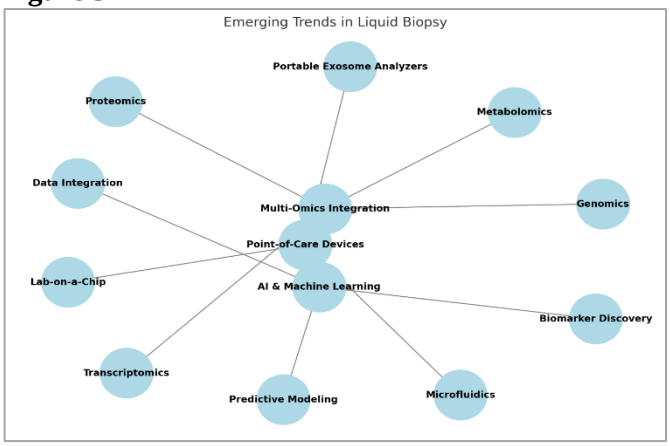
ExoDx Pancreas Test

The ExoDx Pancreas test is focused on exosomal biomarkers, such as GPC1, for the detection of pancreatic cancer. It is still under development but has potential as a non-invasive early detection tool.

Emerging Trends and Future Directions in Liquid Biopsy for Pancreatic Cancer

The liquid biopsy technologies developed at an unprecedented pace and formed a platform for new breakthrough innovations in diagnosis, prognosis, and treatment of pancreatic cancer. Several emerging trends and future directions are pursued to overcome the current limitations and broaden the utility. Such includes the advancements of artificial intelligence and machine learning techniques in multi-omics, point-of-care diagnostic devices, advanced personalized medicine, and many more. These are the enhancements harmonized with these and have been refined in terms of the precision, accessibility, and effectiveness of liquid biopsy in the management of pancreatic cancer.

Figure 3



Multi-Omics Approaches

Actually, one of the most interesting trends in liquid biopsy is a kind of multi-omics approach, where all kinds of different types of molecular data are considered simultaneously-about which, fondly called multiple omics-at the same time-genomics, transcriptomics, proteomics, epigenomics, and metabolomics. Such an holistic approach lets one see tumor biology integrally, thereby enhancing diagnostic as well as prognostic capability of liquid biopsy.

Genomics and Transcriptomics

For example, through integrating genomic data, such as mutations in KRAS, TP53, and CDKN2A, with transcriptomic data, such as the expression levels of tumor-specific microRNAs, a better biomarker of pancreatic cancer can be identified (Wan et al., 2017). For example, it is possible to detect alterations in DNA at both genetic and transcriptional levels by integrating ctDNA analysis with exosomal RNA profiling, thus making the case much more sensitive and specific.

Proteomics and Metabolomics

Proteomic and metabolomic profiling of exosomes and circulating proteins can provide insights into post-transcriptional modification and metabolic changes in tumors. For instance, proteomic biomarkers such as genomic markers glypican-1 (GPC1) in exosomes improve the early detection and risk stratification of pancreatic cancer (Melo et al., 2015).

Multi-Omics in Clinical Applications

With increased detail in the information from more multi-omics approaches on how the mechanism of tumor heterogeneity works to create drug resistance, multi-omics data becomes a handy tool to find new targets and biomarkers for novel treatments towards precision oncology in pancreatic cancer (Siravegna et al., 2017).

Biomarkers and their Analyses through Artificial Intelligence and Machine Learning

Liquid biopsy technologies produce highly complex data, and the complexity of this processing calls for highly powerful tools in computation in order to analyze and interpret it. Areas in AI and ML have been found to be important tools in the domain.

Biomarker Discovery

This research can be considered an avenue through which AI and ML algorithms are sifting through vast databases for the hope of discovering a new biomarker or patterns that can be unattainable through other more traditional statistical approaches. For instance, pancreatic cancer microRNA signatures can be discovered via machine learning models, examples of which appear from the outcomes of a paper on exosomal RNA data as reported by Wang et al. in 2015.

Predictive Modeling

AI-based models combining ctDNA, CTCs, and exosomes can predict the disease progression, treatment response, and risk of recurrence. Supervised learning is inferred in classifying patients on their molecular profile and clinical outcome to thus enable planning for individualized treatments (Cohen et al., 2017).

Data Integration and Interpretation

Multiscale omics data need advanced computational frameworks for the high-throughput analysis of high-complexity data. AI methods can further process high-dimensional datasets, find significant correlations, and derive actionable insights while saving considerable amounts of time and effort that could be involved in analyzing data (Groot et al., 2021).

Point-of-Care Devices for Real-Time Diagnostics

The recent trends have been such that liquid biopsy research was portable and user-friendly point-of-care diagnostic devices, which of quite significant value for real-time diagnosis of which value the resource-limited setting might benefit as such settings might not have the infrastructure of the more advanced laboratory.

Challenges and Opportunities

Indeed, the future of liquid biopsy looks bright, but this main issue is here: standardization of methodologies, low abundance of biomarkers, and high costs. All these can be addressed only through collaboration between researchers, clinicians, and industry stakeholders. The integration with telemedicine and wearables is going to lead to exciting, new ways toward further developments regarding access and usability. Finally, nanotechnology and molecular biology are going to accelerate the application in clinics of even more sensitive and specific liquid biopsy assays.

CONCLUSION

Pancreatic cancer generally occurs in its later stages and advances so quickly, it still remains a very aggressive, not easily diagnosed, and hard-to-handle malignancy, with no strong, early diagnostic agents available. Liquid biopsy is one of the newest more revolutionary approaches currently being developed and represents an alternative tool that minimally invasive and occurs in real-time as compared to the traditionals used up until now for diagnosis and follow-up of the process. Liquid biopsy makes available valuable tumor-derived biomarkers, through the analysis of circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), exosomes, and their other forms.

Liquid biopsy has vast promises for the early diagnosis, assessment of prognosis, and even monitoring of the treatment processes in patients having pancreatic cancer with a need for improvement of their outcomes. Liquid biopsy is now considered a paradigm shift in the management of pancreatic cancer. In contrast, traditional tissue biopsies are invasive procedures that take a lot of time and often suffer from sampling bias. Liquid biopsy provides for a dynamic and detailed view of the molecular profile of a tumor. It uses genetic mutations of ctDNA including KRAS, TP53, CDKN2A, and SMAD4, besides exosomal tumor-derived RNA and proteins in detecting the disease even at the asymptomatic level (Melo et al., 2015; Zill et al., 2015). This is critical in pancreatic cancer where even an earlier stage of diagnosis makes a difference in the possibility for survival.

Liquid biopsy goes beyond diagnostics since it can capture all the molecular heterogeneity in the tumor, which holds critically important prognostic information including tumor burden, metastatic potential, and likelihood of disease recurrence. The monitoring ability of liquid biopsy allows clinicians to monitor the efficacy of the treatment and monitor emerging resistance mutations early. This enables them to make timely changes to therapeutic strategies (Bettegowda et al., 2014). This kind of information is invaluable for tailoring such individualized treatment plans with patients who are made to receive the best therapies known within their unique tumor profiles.

Probably, one of the areas holding the most promise for revolutionizing early detection and surveillance of disease is through liquid biopsy. Pancreatic cancer is very late in detection with very minimal treatment options to be given as well as not very good survival prognosis. Such a problem can be overcome because this technology of liquid biopsy assist researchers in the detection of such specific biomarkers produced by a tumour in blood or body fluids. That is, it can detect diseases at a further, treatable stage (Wan et al., 2017). Such sensitivity and specificity of differentiating the pancreatic cancer patient from her healthy counterparts has been demonstrated in the GPC1-positive exosomes and circulating miR-21, to be of high value for the diagnostics at early disease stages (Melo et al., 2015; Wang et al., 2015).

Further, liquid biopsy allow monitoring dynamics within the tumor longitudinally. Serial sampling allows a clue about the dynamics of ctDNA level changes, and therefore, real-time monitoring of the effectiveness of therapy and disease course. That is a great asset in the evaluation of minimal residual disease and early recurrence as timely intervention is key to long-term survival (Cohen et al., 2017). This advance makes it clear up to which extent liquid biopsy may raise the standard of care for pancreatic cancer patients.

Despite the great promise of liquid biopsy, it remains in its infancy stages of clinical implementation, and much needs to be done for this to become a reality. Biomarkers such as ctDNA and CTCs are present at extremely low concentrations in the blood stream,

especially in early pancreatic cancers. Detection technologies include ultra-deep sequencing and droplet digital PCR, which have improved sensitivity although further studies needed to make them more mainstream and optimize the methods (Mouliere et al., 2018).

The second area of significant challenge is standardization. The sampling process, sample processing, and analysis may differ; therefore, it could impact the reproducibility and comparability of the results. Standardized liquid biopsy workflows very significant in the formation of a protocol that hence standardize across the different laboratories and settings in a clinical setting (Alix-Panabières & Pantel, 2021). The integration of liquid biopsy data into traditional diagnostic techniques and multi-omics approaches requires computational tools and pipelines for complex data set interpretation and management.

Another factor hindering the widespread use of liquid biopsy is that its advanced technologies, such as next-generation sequencing and microfluidic platforms, come at a very high cost. The creation of low-cost, scalable solutions and point-of-care diagnostic devices be very crucial in enabling the use of liquid biopsy, particularly in resource-poor settings (Visser et al., 2018). In such a scenario, there is an urgent need from the researches, clinicians, and industry for joint efforts towards overcoming these challenges as well as in transforming liquid biopsy innovation into routine clinical practice much quicker.

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