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Revolutionizing Brain Cancer Diagnosis: A Review Of Liquid Biopsies And ctDNA Analysis

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Abstract

Brain tumors are rare yet highly complex malignancies that pose significant diagnostic and therapeutic challenges. Liquid biopsy (LB) has emerged as a novel, non-invasive approach to detect and monitor brain tumors by analyzing biomarkers such as circulating tumor DNA (ctDNA) in bodily fluids. This study aims to evaluate the effectiveness and limitations of ctDNA-based liquid biopsies in diagnosing and monitoring brain tumors, with a focus on gliomas and glioblastomas, and to assess how current techniques can be improved. It reviewed 11 original articles from PubMed and Google Scholar, selected based on the criteria of using ctDNA as the main biomarker for cancer detection. The findings underscore ctDNA's promise for brain tumor diagnosis and monitoring in liquid biopsies but highlight the need for more sensitive methods for early detection. Future advancements should focus on multimodal approaches combining ctDNA analysis with additional biomarkers, such as extracellular vesicles (EVs), and leveraging AI-enhanced imaging technologies like MRI for improved risk prediction and classification. Further, standardized protocols across laboratories are essential to ensure consistent and reliable results, ultimately improving outcomes in brain tumor diagnosis and management.

Keywords: Liquid Biopsy, Circulating tumor DNA, Brain Tumors

Introduction

Brain cancer remains one of the biggest challenges in oncology due to its aggressive nature and complex characteristics. The unique anatomical, physiological, and pathological features of brain tumors, combined with their high mortality rates, contribute to the difficulty in effectively treating these cancers (Chakroun et al., 2017). The situation is further exacerbated by poor prognosis and high relapse rates, which create additional hurdles for treatment outcomes in patients (Shah et al., 2018). Brain tumors, characterized by abnormal cell growth within the brain or surrounding structures, can be classified as either benign (non-cancerous) or malignant (cancerous). Malignant tumors are particularly aggressive, exhibiting rapid, uncontrolled growth and the ability to metastasize to other areas of the body. Unlike benign tumors, their invasive nature makes them more dangerous and challenging to treat. Among primary malignant brain tumors, gliomas and glioblastomas are the most prevalent, with an estimated annual incidence of approximately 17,000 new cases in the USA (Omuro et al., 2013). Other brain tumors, such as meningiomas and chordomas, also occur; however, this research paper will focus specifically on gliomas and glioblastomas.

Genetic mutations play a crucial role in the development and progression of these tumors. In glioblastomas, commonly mutated genes include *PTEN*, *TP53*, and *EGFR*, while in gliomas, *IDH1*, *TP53*, and *ATRX* are frequently altered. As Crespo et al. (2015) explain, "different genetic alterations and genetic pathways appear to distinguish primary (e.g., EGFR amplification) versus secondary (e.g., IDH1/2 or TP53 mutation) GBM." These genetic alterations are significant, as they contribute to differences in tumor behavior, age of diagnosis, and survival outcomes. Given the high mortality rates and the significant impact on patients' quality of life, it is essential to improve our understanding of these tumors' structures and functions. This knowledge will help advance the development of targeted, tumor-specific treatments. Furthermore, reviewing current treatment options and exploring innovative therapeutic approaches is critical in addressing the challenges associated with gliomas and glioblastomas.

Diagnosis of Brain Tumors

Despite advancements in medicine, brain tumors are traditionally monitored through invasive methods such as imaging, surgery, and tissue biopsies. For instance, tumor detection and segmentation using MRI (Magnetic Resonance Imaging) is a common diagnostic tool (Abd-Ellah et al., 2019). However, tissue biopsies and surgical resections carry significant risks, including complications from surgery and the potential for lesions in critical areas such as the brain stem (Afflerbach et al., 2024). Due to the invasive nature of these traditional diagnostic approaches, liquid biopsies have emerged as a promising, non-invasive alternative for tumor diagnosis, profiling, and monitoring responses during treatment.

Liquid biopsies analyze biomarkers present in bodily fluids, such as blood, urine, or cerebrospinal fluid. These biomarkers include circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), and extracellular vesicles (EVs), among others. By detecting these components, liquid biopsies provide valuable insights into the molecular characteristics of

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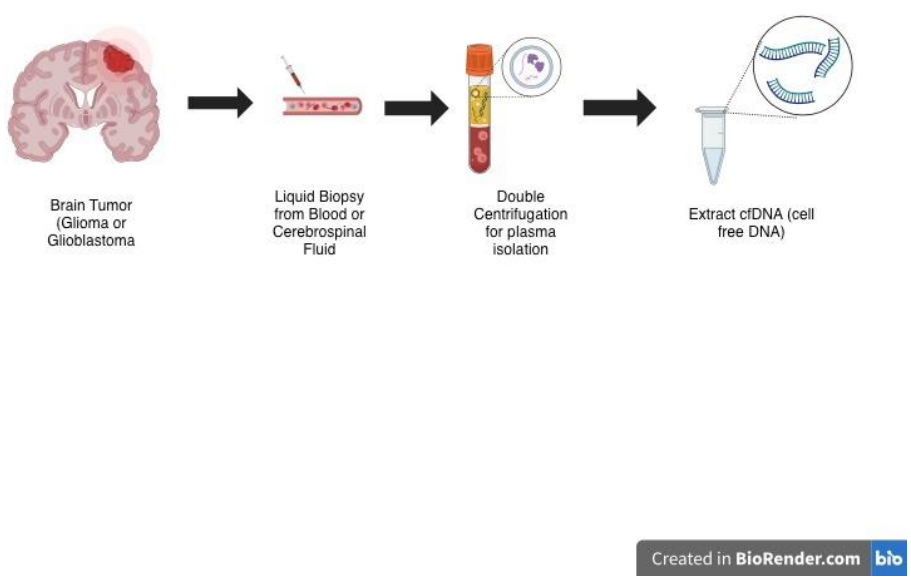
tumors without requiring invasive surgical procedures. In the context of brain tumors, this paper focuses specifically on cell-free DNA (cfDNA) fragments derived from tumor tissue in blood. Among these, circulating tumor DNA (ctDNA) serves as a key biomarker; ctDNA fragments, which are released by tumor cells into the bloodstream, enable the detection of specific tumor features, including genetic mutations, duplications, and deletions within the genome. The majority of genome alterations in cancer are preserved in cfDNA, which are fragments of DNA released by cancer cells into bodily fluids such as blood and cerebrospinal fluid (Bronkhorst et al., 2023). This method is particularly advantageous for brain tumors, as it facilitates the identification and monitoring of tumor-specific alterations in a non-invasive manner, overcoming some of the challenges posed by traditional diagnostic methods.

Figure 1

Process of ctDNA Extraction in Brain Tumor Analysis Using Liquid Biopsies



In brain tumors, specifically gliomas or glioblastomas, blood or cerebrospinal fluid (CSF) surrounding the tumor is taken for molecular analysis. After blood is taken, the plasma is isolated using a double centrifugation technique. The cfDNA must be extracted from the plasma to finally analyze the cell-tumor DNA (ctDNA) within the cfDNA using digital PCR (dPCR) or next generation sequencing (NGS) based approaches.

Biology of cfDNA

The biology of cfDNA is crucial as it plays a key role in the liquid biopsy analysis process. For example, tumors release "various components into the bloodstream" of cfDNA, including "microRNAs (miRNAs), circulating tumor DNA (ctDNA), circulating tumor cells (CTCs), proteins, extracellular vesicles (EVs) or exosomes, metabolites, and other factors" (Seyhan et al., 2024). Key components of cfDNA that influence the liquid biopsy extraction process include the size of ctDNA-cfDNA fragments, the tissue of origin (which affects fragmentation patterns), and the presence of targeted mutations.

Advantages of Liquid Biopsies

Compared to tissue biopsies or surgery, liquid biopsies are less expensive, non-invasive, and more comfortable for patients. Additionally, they provide a more comprehensive overview of the tumor's structure, facilitating the development of personalized treatment plans. In the context of brain tumors, liquid biopsies offer a significant advantage by enabling the non-invasive analysis of tumor changes. For instance, in glioblastomas, where tumor accessibility is often limited, liquid biopsies provide an opportunity to investigate specific genetic and epigenetic alterations (Trivedi et al., 2023). This approach reduces the risks associated with invasive procedures, minimizes the impact on patients' quality of life, and simplifies data collection.

Blood Brain Barrier

While liquid biopsies present promising advancements as a diagnostic approach, there are notable limitations to their use. Because liquid biopsies are relatively new, tumor-derived biomarkers from blood or cerebrospinal fluid (CSF) are not yet utilized in routine clinical practice (Rincon-Torroella et al., 2022). Additionally, the blood-brain barrier (BBB) poses a significant challenge. The BBB separates the brain's blood vessels from surrounding tissue and neurons, regulating the movement of substances between the bloodstream and the brain while preventing harmful toxins from entering. Although essential for maintaining a stable environment, the BBB also complicates treatment by blocking the delivery of therapeutic drugs to the brain (Wu et al., 2023).

In gliomas and glioblastomas specifically, the BBB can become disrupted as tumors grow and invade surrounding tissues. This disruption allows some tumor-derived materials to enter the bloodstream; however, it also reduces the sensitivity of liquid biopsies, as not all tumor markers are detectable in blood. Despite this limitation, ongoing research is exploring strategies to overcome the challenges posed by the BBB. One promising method involves the use of focused ultrasounds to temporarily open the barrier. For example, enhancing the presence of tumor biomarkers in blood and CSF via "brain-blood barrier (BBB) disruption with MRI-guided focused ultrasound (MRgFUS)" is a very compelling strategy for "future management of brain tumor patients" (Rincon-Torroella et al., 2022).

Methods

Table 1

Summary on the use of circulating tumor DNA (ctDNA) and other biomarkers in liquid biopsy for brain tumor detection and monitoring

First Author and Publication Year	Research Question	Biomarker	Targeted Gene	Method	Type of Blood Collection	Tumor Stage	Number of Cases
Martínez-Ricarte F., 2018	Can CSF ctDNA be used as a liquid biopsy to diagnose diffuse gliomas?	CSF ctDNA	IDH1, IDH 2, TP53, ATRX, TERT	Droplet digital PCR, and exome sequencing	CSF collected by lumbar puncture	IV	20 clinical specimens
Aleksandra Majchrzak -Celínska, 2013	Are DNA methylation-based biomarkers a promising diagnostic technique for cancer in the central nervous system?	ctDNA	MGMT, RASSF1A, p15INK4B, and p14ARF gene s	Methylation-specific polymerase chain reaction (PCR)	Sodium iodide/ glycogen method	N/A	33

Florent Mouliere, 2018	Are gliomas easier to detect using CSF for liquid biopsies?	ctDNA	EGFR, PTEN, IDH1	Shallow whole genome sequencing	N/A	Primary glioma	13
Attila A. Seyhan, 2024	Can liquid biopsies provide dynamic monitoring of GBM characteristics?	ctDNA, miRNA, CTCs, EVs	Various tumor-specific genes	Non-invasive blood sampling for collection analytes	Circulating analytes from blood	Grade IV GBM	N/A
David E. Piccioni, 2019	What are the detection rates of ctDNA and genetic alterations in primary brain tumors using liquid biopsy?	ctDNA, cfDNA alterations	TP53, EGFR, IDH1, NF1	NGS Assay	Plasma Collection	All stages, including GBM Grade IV	419 (211 with alterations)
	Is plasma cfDNA an effective	cfDNA	TP53, EGFR, ERBB2, NF1	Next-generation	Plasma	Newly diagnosed GBM	42 (newly diagnosed GBM)

Stephen J. Bagley, 2020	noninvasive biomarker for prognosis in glioblastoma (GBM)?			sequencing (NGS)			
Stephen J. Bagley, 2021	Is plasma cfDNA concentration associated with survival in patients with IDH wild-type glioblastoma?	cfDNA	MGMT Promoter	qPCR	Plasma	IDH wild-type GBM	62
Francisco Martínez-Ricarte, 2018	Can ctDNA in cerebrospinal fluid be used to subclassify diffuse gliomas?	ctDNA	IDH1, IDH2, TP53, ATRX, TERT, H3F3A, HIST1H3B	Targeted exome sequencing, ddPCR	CSF (Cerebrospinal Fluid)	Diffuse gliomas	648 gliomas, 20 clinical cases

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Jordan J. Jones, 2024	Can IDH1, TERTp, and EGFRvIII mutations in plasma ctDNA be reliably detected for glioma liquid biopsy?	ctDNA	IDH, TERTp, EGFRvIII	ddPCR	Plasma	Glioma (all grades)	110 patients (359 plasma samples)
Elisa Izquierdo, 2021	Can ctDNA in CSF, plasma, serum, or cystic fluid provide tumor-specific information for pediatric HGG/DMG?	ctDNA	24 genes: ALK, BCOR, BEND2, BRAF, etc.	ddPCR	CSF and plasma	gliomas- pediatric high grade and diffuse midline	32- liquid biopsy cohort 41- plasma specimens

Yuxuan Wang, 2015	Can CSF-ctDNA detect tumor-specific mutations?	ctDNA	IDH1, TERT, NF2, PTCH1	Targeted and genome-wide sequencing	CSF	High grade gliomas (I-IV)	35

Table 1 highlights the growing importance of ctDNA in both CSF and plasma for brain tumor detection. CSF-derived ctDNA shows strong diagnostic relevance, especially for tumors that are difficult to access surgically. For example, Martínez-Ricarte et al. (2018) and Wang et al. (2015) demonstrated the ability of CSF ctDNA to detect key mutations such as *IDH1*, *TP53*, and *TERT* using digital PCR and sequencing methods. These findings support CSF as a more direct and mutation-rich source of ctDNA, particularly in diffuse or midline gliomas. Plasma-derived ctDNA, while more accessible, presents some sensitivity limitations due to the blood-brain barrier. Still, it has shown utility in prognosis and monitoring. Bagley et al. (2020, 2021) used plasma cfDNA to assess prognosis in glioblastoma, with findings indicating that cfDNA concentration correlates with patient survival. Piccioni et al. (2019) further validated plasma ctDNA as a diagnostic tool by detecting common alterations like *TP53*, *EGFR*, and *IDH1* across a large sample of 419 brain tumor cases.

In addition to point mutations, newer studies are expanding liquid biopsy applications through emerging techniques. Majchrzak-Celińska (2013) explored DNA methylation-based biomarkers, identifying *MGMT* and *RASSF1A* as potential markers using methylation-specific PCR. Mouliere et al. (2018) highlighted shallow whole-genome sequencing as a method for detecting structural alterations, particularly in CSF samples. Lastly, broader biomarkers

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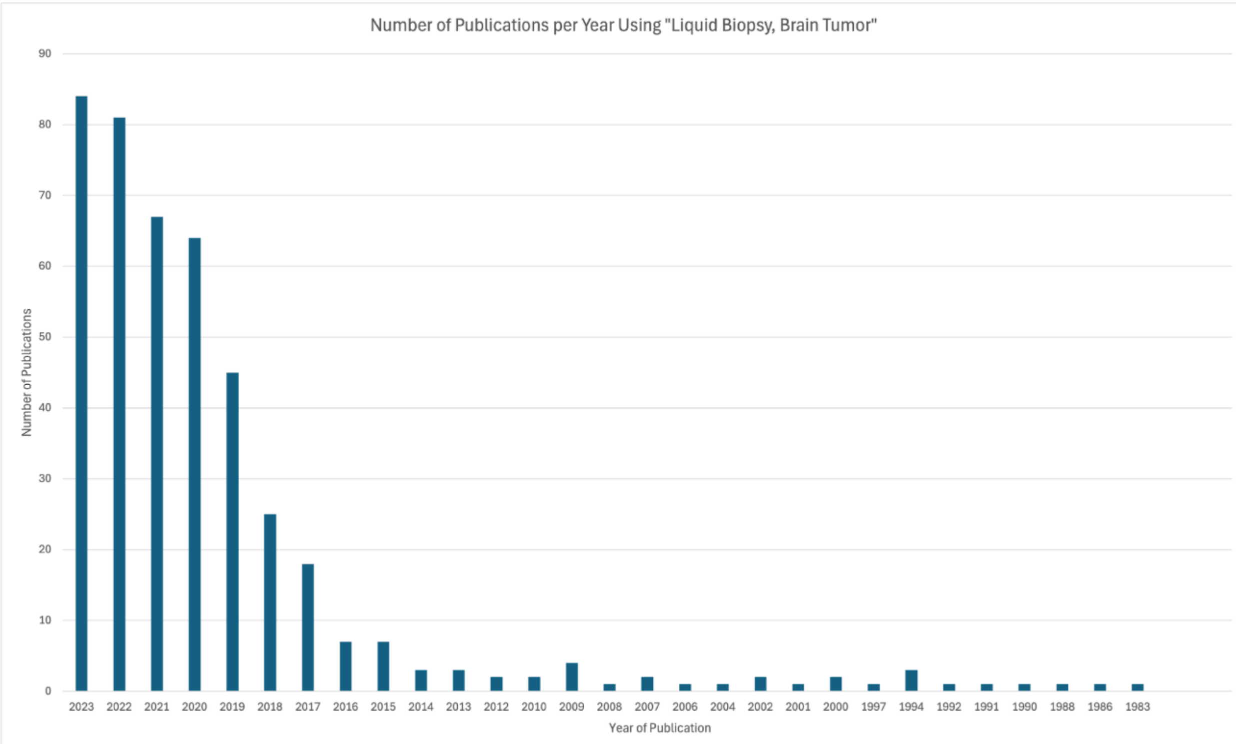
involving miRNAs, extracellular vesicles (EVs), and circulating tumor cells (CTCs), as shown by Seyhan et al. (2024) and Izquierdo et al. (2021), signal a shift toward multi-analyte liquid biopsy approaches, especially for complex tumors like pediatric high-grade gliomas. Together, these studies show how liquid biopsies can complement or even replace more invasive diagnostic tools in brain tumor management, though standardization and sensitivity remain major areas for improvement.

Results

This research highlights the utility of circulating tumor DNA (ctDNA) as a promising biomarker for the early detection and monitoring of brain tumors. However, early detection remains challenging due to the low ctDNA fraction in liquid biopsy samples. Glioblastomas and Gliomas are some of the most commonly studied and aggressive forms of brain cancer. Despite advancements, more clinical analytic studies are required to improve detection methods and validate ctDNA as a reliable diagnostic tool.

Figure 2

Visual Representation of The Number of Research Publications on "Liquid Biopsy, Brain Tumor" Each Year From 1983-2023



As shown in the figure, the number of publications spiked, which can be attributed to the development of Next-generation sequencing (NGS). This breakthrough in sequencing technology represented a significant leap from traditional Sanger sequencing. Due to NGS, throughput DNA sequencing became faster and more efficient, allowing multiple DNA fragments to be sequenced in parallel. Overall figure 2 highlights the importance of modern NGS sequencing techniques. *2009 was the year of NGS (next-generation sequencing) development which set the stage for more research on liquid biopsies. Further, 2012 was the year of the first publication of NGS using non-invasive prenatal testing with cfDNA analysis.

Discussions

This study aimed to explore the role of circulating tumor DNA (ctDNA) and other liquid biopsy biomarkers in the diagnosis and monitoring of brain tumors, specifically gliomas and glioblastomas. By synthesizing current findings, the research highlights the potential and challenges associated with integrating advanced molecular techniques, such as next-generation sequencing (NGS), with liquid biopsy methods to improve early detection, classification, and disease monitoring in brain tumor patients.

Summary of Main Results

The results of this paper highlight the growing importance of ctDNA as a biomarker, particularly in CSF and plasma samples, in liquid biopsies for brain tumor diagnosis. NGS has revolutionized liquid biopsy by enabling high-throughput and precise mutation detection in ctDNA and cfDNA. It is particularly effective in detecting alterations in genes such as EGFR, IDH1, and MGMT. In brain tumors, where tissue biopsies are often invasive and risky, liquid biopsy using NGS offers a non-invasive alternative to capture tumor-specific information. Additionally, it supports real-time monitoring and individualized treatment strategies, especially in glioblastomas (GBM). However, challenges in ensuring consistent sample quality and interpreting data limit its widespread application. Further, while advances in NGS and other sequencing methods have improved mutation detection in genes such as IDH1, TERT, and TP53, the low tumor fraction in ctDNA poses significant challenges for early detection.

Pediatric Brain Tumors

While most research on ctDNA has focused on adult brain tumors such as glioblastoma, extending these methods to pediatric cases is equally important. Brain tumors in children differ biologically and clinically from those in adults, which shapes how liquid biopsy strategies can be applied. Brain tumors in pediatric and adult patients differ significantly in their biology, genetic profiles, and clinical behavior. These differences play a crucial role in the application of liquid biopsies for diagnosis and monitoring and understanding them is essential for tailoring liquid biopsy techniques to specific patient populations.

First, pediatric and adult brain tumors exhibit distinct genetic and prognostic profiles, which have critical implications for liquid biopsy approaches. In adult patients, "IDH mutations serve as positive prognostic markers" with the most significant prognostic relevance. Furthermore, features such as TERT promoter mutations or EGFR alterations can "upgrade IDH-wildtype astrocytomas to glioblastoma" (Śledzińska et al., 2021). In contrast, different developmental mutations affect pediatric patients. For instance, "H3F3A alterations" are among the most important markers in pediatric patients, "predicting poor outcomes in tumors such as diffuse midline gliomas (DMGs)" (Śledzińska et al., 2021).

These biological differences necessitate age-specific liquid biopsy approaches to improve diagnostic and therapeutic outcomes. Further, children's tumors are generally less infiltrative but can be more anatomically diffuse, often involving critical structures like the brainstem. Adult tumors, especially glioblastomas, are highly invasive and tend to involve multiple regions of the brain, potentially increasing the shedding of tumor-derived DNA into circulation (Madlener et al., 2020).

Tumor Microenvironment

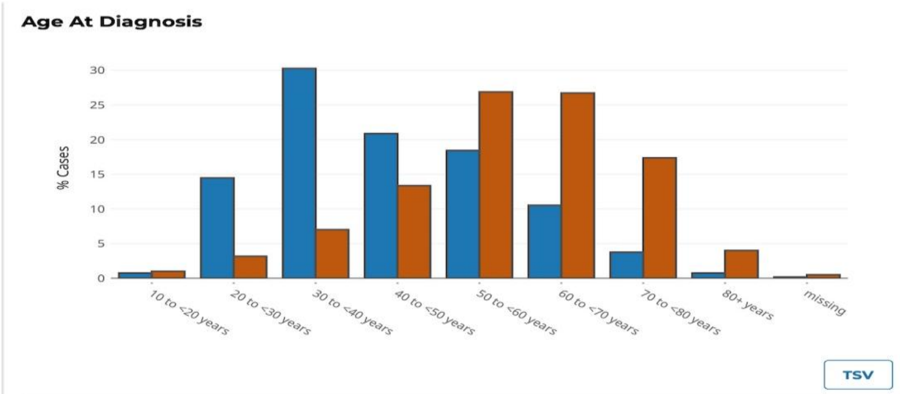
The tumor microenvironment in children is likely to have less extensive abnormalities in the glioblastomas than in adults. This difference can influence the release of cfDNA and ctDNA into the bloodstream or cerebrospinal fluid. For example, "pediatric tumors, especially those near CSF reservoirs, may shed more detectable ctDNA into the CSF than into the plasma", whereas adult tumors with a more "disrupted blood-brain barrier" might release ctDNA into both compartments (Tripathy et al., 2023). Studies have shown that "CSF-derived ctDNA better reflects driver gene mutations in glioblastoma patients compared to plasma-derived ctDNA." This outcome is partially due to the "higher concentration of ctDNA in CSF" and its "low levels in plasma caused by the blood-brain barrier." However, the clearance of ctDNA in the bloodstream and CSF remains poorly understood and warrants further investigation (Madlener et al., 2020).

Clinical Implications

The biological and microenvironmental differences influence the utility of liquid biopsies in clinical settings. For pediatric patients, CSF-based liquid biopsies are emerging as a minimally invasive alternative to tissue biopsies, especially for tumors in surgically inaccessible locations. In adults, plasma-based liquid biopsies hold promise for monitoring tumor progression and treatment response, although sensitivity remains a challenge. Advances in detection methods, such as droplet digital PCR (ddPCR) and next-generation sequencing (NGS), are improving the feasibility of these approaches for both populations (Tripathy et al., 2023).

Figure 3

Visual Representation of Age at Diagnosis for Glioma and Glioblastoma Patients Across Two NIH GDC Cancer Portal Cohorts



Age At Diagnosis	# Cases S_1	% Cases S_1	# Cases S_2	% Cases S_2
10 to <20 years	4	0.75 %	6	1.00 %
20 to <30 years	77	14.47 %	19	3.17 %
30 to <40 years	161	30.26 %	42	7.01 %
40 to <50 years	111	20.86 %	80	13.36 %
50 to <60 years	98	18.42 %	161	26.88 %
60 to <70 years	56	10.53 %	160	26.71 %
70 to <80 years	20	3.76 %	104	17.36 %
80+ years	4	0.75 %	24	4.01 %
missing	1	0.19 %	3	0.50 %

These graphs were taken from 2 different cohorts in the NIH GDC Cancer Portal: gliomas and glioblastomas. From this figure, the orange is the glioblastoma cohort, and the blue is the glioma cohort. As shown above, the age of diagnosis varies between the type of tumor; On average, gliomas get diagnosed at earlier ages, while glioblastomas are diagnosed in later stages of life. The differences can be attributed to a variety of factors, so more research must be done centering adult vs pediatric brain tumors.

Tumor Informed vs Tumor Naïve Approaches

Two key methodologies have emerged in the use of ctDNA: tumor-informed and tumor-naïve approaches. Tumor-informed strategies utilize prior knowledge from tumor tissue biopsies to design personalized assays targeting specific mutations. These methods demonstrate higher sensitivity for detecting minimal residual disease (MRD) and monitoring disease progression. In contrast, tumor-naïve approaches analyze ctDNA without prior tumor-specific information, relying on broader genomic or epigenomic signatures. While these are more versatile and easier to implement, they are less sensitive for certain clinical applications, such as early relapse detection.

Remaining Challenges

Despite significant progress, early detection of brain tumors using liquid biopsy remains challenging. The primary limitation lies in the low tumor fraction of ctDNA in both CSF and plasma, which compromises sensitivity. This is particularly problematic for small or early-stage tumors. Additionally, there is considerable heterogeneity in laboratory protocols, such as cfDNA extraction and quantification methods, further complicating the reliability of results across studies. Therefore, there’s a pressing need for “widely applicable standards” in cfDNA analysis that “include all preanalytical steps from blood draw to analysis” (Greytak et al., 2021). To overcome current limitations, a multimodal diagnostic approach integrating ctDNA with advanced imaging techniques such as “optimized preanalytics” and “machine learning”, for example, can significantly improve the accuracy of tumor detection and “sensitivity of ctDNA assays” (Bronkhorst et al., 2023). For the liquid biopsy to be widely adopted in clinical settings, standard operating procedures (SOPs) must be developed and consistently applied across laboratories. Standardized methods for cfDNA collection, processing, and analysis are critical to ensure reproducibility and reliability of results.

To conclude, while the use of ctDNA and liquid biopsy holds immense promise for brain tumor diagnosis and monitoring, significant challenges remain. A shift toward multimodal approaches that integrate molecular and imaging biomarkers, coupled with standardized protocols, will be crucial in overcoming current barriers. By addressing these gaps, this field can move closer to achieving precision diagnostics and improving outcomes for brain tumor patients.

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