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Targeted liquid biopsy for brain tumors

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ABSTRACT

Cerebrospinal fluid (CSF) is a promising source of molecular biomarkers in the detection and monitoring of brain malignancies. Unlike peripheral blood, where biomarker detection is hindered by the blood-brain barrier and the complex nature of biofluids, CSF offers a more direct and enriched source of tumor-derived markers, including circulating tumor DNA (ctDNA), microRNA (miRNA), proteins, and extracellular vesicles (EVs). These biomarkers, originating from brain tumor cells, are often more concentrated in CSF than in peripheral blood due to the proximity of CSF to the central nervous system (CNS). The presence of ctDNA in CSF is notably higher than in plasma, making CSF an advantageous medium for liquid biopsy in brain tumor patients. Traditional liquid biopsy approaches relying on peripheral venous blood samples often face challenges in detecting low concentrations of tumor-derived biomarkers. The direct contact of CSF with the CNS minimizes background noise, potentially enhancing the accuracy and sensitivity of diagnostic assays. Despite the barriers posed by the blood-brain barrier and other physiological factors that limit biomarker levels in the systemic circulation, CSF's unique position within the CNS allows for more effective biomarker collection. While peripheral blood remains the standard medium for liquid biopsy in oncology, the proximity of CSF to brain tumors suggests it may offer superior diagnostic capabilities. Emerging evidence from non-CNS malignancies indicates that collecting biofluids closer to the tumor site can significantly improve biomarker detection. Although this approach has been well-documented in other solid tumors, its application to CNS malignancies remains underexplored. This study hypothesizes that targeted blood sampling from CNS tumor-draining veins could similarly enhance biomarker detection, thereby increasing the sensitivity and efficacy of liquid biopsy techniques in diagnosing and monitoring brain malignancies.

1. Introduction

Brain tumors are significant contributors to cancer morbidity and mortality in children and adults. These tumors account for about 20–30 % of cancer-related deaths [1]. The average annual age-adjusted incidence rate of all central nervous system (CNS) tumors in the United States was 23.79 per 100,000 persons between 2013 and 2017 [2]. The most common malignant primary brain tumor is glioblastoma, with a dismal five-year survival rate of less than 7 percent [3]. The brain is also a secondary site for metastasis from primary tumors located outside the CNS, with a combined incidence rate of approximately 9–17 % across all cancer types. Among these, lung cancer, breast cancer, and melanoma are the most prevalent cancers that metastasize to the brain [4].

Diagnosing brain tumors relies on non-invasive neuroimaging modalities, e.g., magnetic resonance imaging (MRI) and computerized tomography (CT), followed by surgical tissue biopsy for histological confirmation and genetic profiling [5]. However, interpreting changes in neuroimaging, especially post-treatment (e.g., pseudo-progression), can be challenging [6]. Moreover, tissue biopsy requires surgery and may result in complications like bleeding or infection [7]. Tracking tumor evolution, treatment response, and recurrence generally requires repeated biopsies, which are not always practical. Tumor biopsies may sometimes be challenging when tumors are in complex sites, or patients are too fragile to tolerate invasive procedures [8].

Minimally invasive biofluid-based liquid biopsies are rapidly emerging techniques for diagnosing, assessing the prognosis, monitoring

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treatment response, genetic profiling for personalized therapy, asymptomatic screening, and detecting the residual disease of tumors [9–11]. It refers to the analysis of tumor-derived biomarkers, including circulating DNA (ctDNA), circulating RNA, micro-RNA, extracellular vesicles (EVs), circulating tumor cells (CTCs), and platelets in different biofluids such as blood, saliva, urine, and cerebrospinal fluid (CSF) [12]. Brain tumor-derived biomarkers are generally low in abundance and detected in limited patients, which makes liquid biopsy challenging in clinical practice [13–15]. One of the challenges to brain tumor-derived biomarker detection is the permeability of the blood-brain barrier (BBB), which limits the release of these biomarkers into the peripheral circulation, resulting in low detection sensitivity of this technique [16, 17]. The challenges also include dilution of tumor biomarkers with physiological biomarkers present in peripheral blood, the high volume of blood [18], and the rapid clearance of biomarkers by the liver, spleen, kidney, and plasma enzymes [19–21]. Therefore, the targeted approaches, i.e., CSF and blood from a tumor-proximal vein for liquid biopsies, can bypass these challenges.

CSF, as a targeted liquid biopsy, offers several advantages [22]: 1) The blood-brain barrier (BBB) does not pose a permeability challenge to the biomarkers of CSF, 2) CSF is in close proximity to tumors or in direct contact with them, resulting in a higher concentration of biomarkers compared to plasma, 3) CSF has low cellularity, which means it contains less noise than plasma, 4) CSF primarily contains biomarkers from the central nervous system (CNS), while plasma contains biomarkers from all organ systems in the body. However, CSF liquid biopsy is more invasive than venipuncture [22]. Moreover, the common complications following a lumbar puncture (LP) are short-term post-procedural headaches along with varying levels of discomfort or pain. Less commonly, complications such as spinal hematoma, especially in patients on anticoagulants or with thrombocytopenia, and infection can occur. In cases where patients have significantly elevated intracranial pressure due to mass effect, edema, or obstructive hydrocephalus, a lumbar puncture carries the risk of cerebral herniation, making it a relative contraindication for the procedure [23]. In this review paper, we discussed how taking targeted liquid biopsies from CSF and blood of tumor-proximal vessels can improve detection sensitivity for CNS tumors.

2. Current diagnostic methods for brain tumors

2.1. Neuroimaging for brain tumors

MRI and computed tomography (CT) are now the primary imaging tools for assessing intracranial lesions, offering detailed visualizations of brain anatomy in a slice-by-slice format. While MRI is preferred for detecting and characterizing brain tumors, CT is better suited for evaluating bone abnormalities and identifying calcifications [24]. At the time of initial diagnosis, it is essential to distinguish brain tumors from benign lesions, which may look similar on MRI scans. Contrast-enhanced MRI is favored because of its superior soft-tissue resolution and wide availability. Diagnosing brain tumors generally relies on conventional MRI, which uses T1-weighted and T2-weighted sequences. However, standard imaging often struggles to differentiate between tumor progression and nonspecific, treatment-related changes, particularly after therapy.

Positron emission tomography (PET), using various radioactive tracers that target metabolic and molecular processes, can provide additional, valuable information, particularly in cases where the diagnosis is unclear [25]. Over the past decade, PET with radiolabeled amino acids has become a crucial diagnostic tool. The Response Assessment in Neuro-Oncology (RANO) working group has recommended integrating amino acid PET imaging with MRI for managing brain tumors.

At the same time, advanced MRI techniques like perfusion-weighted imaging (PWI), diffusion-weighted imaging (DWI), and proton magnetic resonance spectroscopic imaging (MRSI) are being clinically evaluated. These methods can provide important physiological or biochemical insights that go beyond standard MRI [26].

2.2. Stereotactic biopsy for brain tumors

Conventional MRI is an invaluable tool for the initial diagnosis of intracranial tumors. However, it cannot fully determine the extent of these tumors [26]. As a result, a histopathological examination of tissue samples is necessary for accurate diagnosis, prognosis, and treatment planning. These samples are typically obtained through a biopsy or an open resection [27]. Several techniques are available for performing a tissue biopsy. Traditionally, the frame-based stereotactically guided needle biopsy has been considered the gold standard. However, its use is limited by several factors, including the bulkiness of the frame, patient discomfort, the complexity of calculating stereotactic entry points, lengthy procedure times, and the risk of infections at the frame's fixation sites [28,29]. In recent years, frameless techniques that use navigational technologies have gained popularity due to their ease of use and comparable diagnostic yield. However, these techniques pose a higher risk when biopsying deep cranial lesions.

Additionally, robot-assisted stereotactic brain biopsies and endoscopic biopsies for intraventricular and paraventricular lesions are also employed in various clinical settings [28–30]. Despite the differences in these procedures, the complications associated with needle biopsy remain relatively consistent across studies. Intracranial hemorrhage is the most common complication following a needle brain biopsy, but other potential issues include neurological impairments such as transient or permanent deficits, edema, seizures, unconsciousness, and, more rarely, infections [31,32].

3. CSF as targeted liquid biopsy for brain tumors

CSF is a crucial source of potential molecular biomarkers, primarily collected via lumbar puncture (Fig. 1) or surgical procedures near the brain. CSF encompasses a variety of biomarkers, such as circulating tumor DNA (ctDNA), microRNA (miRNA), proteins, and extracellular vesicles (EVs), typically originating from brain tumor cells [33]. Tumor cells often coexist with their surrounding microenvironment, making tumor-related markers more prominent in fluids near the disease site. CSF is generally considered an extension of the extracellular compartment within the CNS, thus serving as a key pathway for brain tumors [34]. The biomarker levels in patients with brain tumors are typically low or undetectable due to the significant barrier posed by the blood-brain barrier, which hinders the release of potential biomarkers into the systemic circulation. However, CSF serves as an excellent reservoir for clinical biomarkers, and a growing number of studies indicate that biomarkers derived from CSF are more abundant than those found in peripheral blood and other sources. For example, ctDNA originating from brain tumor cells is present in higher concentrations in CSF compared to plasma [35]. Additionally, CSF serves as a better source of circulating nucleic acids than plasma in patients with brain tumors. Blood is a complex biofluid containing considerable noise, requiring additional processing steps before conducting any biomarker identification studies. For brain tumors, including metastases, CSF presents a viable option for biomarker analysis due to its proximity to the brain and the cancer. Unlike serum or plasma, which are more complex biofluids, CSF's direct contact with the central nervous system provides a clearer, more distinct biomarker signal, minimizing background noise and potentially enhancing diagnostic accuracy [35].

De Mattos-Arruda et al. identified and characterized ctDNA in CSF of patients with brain lesions, showing it provided a more accurate representation of brain tumor genomic alterations compared to plasma ctDNA. Key actionable gene mutations and copy number alterations (CNA) such as *Epidermal Growth Factor Receptor (EGFR), Phosphatase and Tensin Homolog (PTEN), Estrogen Receptor 1 (ESR1), Isocitrate Dehydrogenase 1 (IDH1), Erb-B2 Receptor Tyrosine Kinase 2 (ERBB2 or HER2), and*

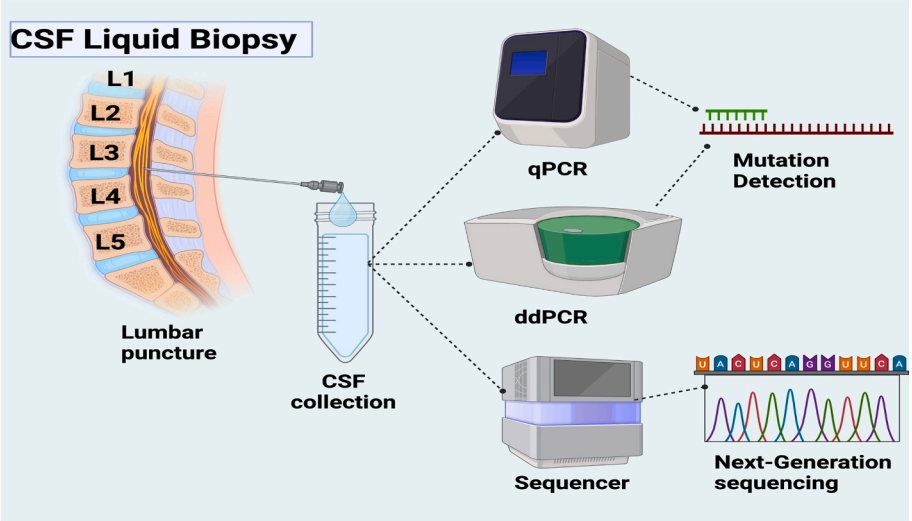

Fig. 1. CSF gives better biomarker detection compared to peripheral blood. CSF is taken mostly via lumbar puncture between L3 and L4 vertebrae. The CSF is then processed for detection or sequencing. qPCR or ddPCR detects the mutated gene, and the sequencer sequences the whole gene related to the tumor.

Fibroblast Growth Factor Receptor 2 (FGFR2) could be identified. CSF ctDNA demonstrated significantly higher sensitivity for detecting CNS genomic alterations and brain tumor-specific mutations, as well as monitoring tumor progression, making it a valuable tool in neuro-oncology [35]. Three gene mutations of brain metastasis with meningeal involvement were present in CSF ctDNA but not in plasma ctDNA [35]. Moreover, mutant allelic frequency (MAF) and detection sensitivity in CSF ctDNA were higher than that of plasma ctDNA [35].

In a study by Ma et al. [36] and colleagues, next-generation sequencing (NGS) was performed on 21 patients with non-small cell lung cancer (NSCLC), including 10 with leptomeningeal metastases (LM) and 11 with brain parenchymal metastases (BPM). Mutations were found in the CSF ctDNA of 20 patients (95.2 %) and in the blood ctDNA of 14 patients (66.7 %). *EGFR* mutations were present in 57.1 % (12/21) of CSF ctDNA samples, compared to 23.8 % (5/21) in peripheral blood ctDNA. The *EGFR* status in CSF ctDNA matched the primary tumor in 16 out of 18 patients (88.9 %). Miller et al. [37] used a high-sensitivity capture-based NGS assay and detected at least one mutation in the cfDNA of CSF in 16 out of 19 (84 %) glioma patients. They found a total of 211 mutations in the CSF. In the plasma of these patients, at least one mutation was detected in three patients, with a total of 35 mutations. The average variant allele fraction (VAF) of these 35 mutations was significantly lower in plasma (0.58 %) compared to 23.96 % in the CSF. Pan et al. [38] used NGS and detected primary tumor alterations in the CSF of 83.8 % (31/37) patients. At least half of the alterations were identified in the CSF of 91.9 % (34/37) of the patients. Compared to plasma from 8 patients, tumor-specific mutations were identified in the CSF ctDNA in all cases (100 %, 8/8) but were only found in the plasma ctDNA in 37.5 % (3/8) cases. Additionally, the median MAF for the four mutations detected in both CSF and plasma was consistently higher in the CSF ctDNA than in the plasma ctDNA.

In a cohort of seven patients with solid brain tumors, mutant alleles were quantified in plasma and CSF cfDNA using ddPCR. Among these patients, one with a primary brain tumor (atypical meningioma) and two with metastatic brain tumors (melanoma brain metastases and lung adenocarcinoma brain metastases) showed no detectable tumor mutations in plasma cfDNA. However, significant concentrations of mutant alleles were present in CSF cfDNA, with levels at 4 % in the atypical meningioma, 7.4 % in the melanoma metastasis, and 0.9 % in the lung adenocarcinoma metastasis. Additionally, in one patient with metastatic brain tumors from colon adenocarcinoma, the mutant allele fraction was notably higher in CSF than in plasma [39]. Similarly, Escudero et al. [40] used ddPCR and found ctDNA in the CSF of 10 out of 13 medulloblastoma patients while in the plasma of only one patient. *ALK Receptor Tyrosine Kinase (ALK)* rearrangement was identified in the CSF of nine out of 11 samples from NSCLC LM (81.8 %) and in the plasma of five out of the 11 samples (45.5 %). Furthermore, the MAF in all CSF cfDNA samples was significantly greater than in the plasma samples [41].

Ying et al. [42] conducted capture-based targeted sequencing in NSCLC LM patients and found that the detection rate from CSF was significantly higher than that from plasma. They also compared the MAF between CSF and plasma, with MAFs of 43.64 % in CSF and 4.58 % in plasma. In total, 280 genomic alterations were identified in CSF samples, mapping to 60 genes, while 137 alterations were identified in plasma samples, mapping to 33 genes. Of these, 197 mutations were unique to CSF, and 54 were unique to plasma. The *EGFR* gene was the most frequently mutated in both mediums, with occurrences in 58.33 % of CSF samples and 44.44 % of plasma samples. Other common alterations included *Tumour Protein P53 (TP53)* mutations, *ALK* fusions, and *Erb-B2 Receptor Tyrosine Kinase 2 (ERBB2)* amplifications, seen in 50 %, 5.6 %, and 1.4 % of CSF samples and in 38.9 %, 5.6 %, and 1.4 % of plasma samples, respectively. A total of 121 copy number variations (CNVs) were detected in CSF, with the majority (81.8 %) being specific to CSF and only 18.2 % shared with plasma. Additionally, *TP53* loss of heterozygosity (LOH) was observed in 41.7 % (30/72) of CSF ctDNA samples, significantly higher than in matched plasma samples 13.9 % (10/72).

In a study conducted by Aldea et al. [43] and colleagues, paired plasma and CSF liquid biopsies were collected from 12 patients, including 11 with leptomeningeal progression and one with brain progression. Among the patients, 11 had known *EGFR* mutations, and one had an *ALK* rearrangement. ctDNA was detected in the Plasma of 6 out of the 12 patients (50 %), while paired CSF samples showed ctDNA positivity in 10 out of the 12 patients (83 %). It is evident from previous studies (Table 1) that CSF provides better detection sensitivity compared to plasma/serum in peripheral blood. CSF overcomes BBB challenges and has direct contact with the tumor microenvironment.

4. Blood from a tumor-proximal vein as a targeted liquid biopsy for brain tumors

In addition to BBB, the challenges to biomarkers from brain tumors include shorter half-life [44–46], clearance by plasma enzymes, liver, kidney, and spleen [19–22], and dilution of the biomarkers in peripheral blood [18]. These limitations prevent liquid biopsy from reaching its full

Table 1
Overview of the studies comparing the sensitivity of biomarker detection between CSF and Plasma.

Tumor type	No. of Patients (N)	Biomarker	Target	Method	Findings	References
GBM, Brain metastasis	N = 12 GBM = 4 BMBC = 4 BMLC = 2	ctDNA	• Targeted sequencing of all exons of 341 cancer genes harbouring actionable mutations. • Targeted sequencing of all exons of 254 genes recurrently mutated in breast cancer and/or related to DNA repair for BMBC.	NGS, ddPCR	MAF and detection sensitivity in CSF ctDNA were higher than that of plasma ctDNA. Three genes mutation of brain metastasis with meningeal lesions were present in CSF ctDNA but not in plasma ctDNA. CSF ctDNA, being high in MAF, was a better biomarker for longitudinal monitoring than plasma ctDNA.	[35]
NSCLC brain metastasis	N = 21	ctDNA	*EGFR, KIT, PIK3CA, TP53, SMAD4, ATM, SMARCB1, PTEN, FLT3, GNAS, STK11, MET, CTNNB1, APC, FBXW7, ERBB4, and KDR*	NGS	Mutations were detected in the CSF ctDNA of 20 out of 21 patients (95.2 %) versus in the plasma ctDNA of 14 out of 21 patients (66.7 %).	[36]
Glioma	N = 85	cfDNA	*ERT, TP53, IDH1, CDKN2A/B, EGFR, ATRX, PTEN, CIC, NF1, PIK3CA, CDK4, PIK3R1, RB1, PDGFRA, SOX2, NOTCH1, KIT, MDM2, SETD2, PTPN11, FUBP1, KMT2D, FAT1, and KDR.*	NGS	211 mutations were detected in CSF cfDNA of 16/19 glioma patients while 35 mutations in the plasma cfDNA of 3/19 patients. The average VAF of the 35 mutations was 23.96 % in CSF and 0.58 % in plasma	[37]
Brain stem glioma	N = 57	ctDNA	*H3F3A, TP53, ATRX, PIK3CA, PPM1D, PDGFRA, HIST1H3B, IDH1, ACVR1, NF1, PTEN, FGFR1, BCOR, EGFR, BRAF, FAT1, SETD2, PMS2, MET, KIT, FGFR3, RGPD3, PIK3R1, BRCA2, MLH1, MSH6*	NGS	Tumor-specific mutations were detected in the CSF ctDNA of 100 % (8/8) of patients and in the plasma ctDNA of 37.5 % of patients. Median MAF for 4 mutations were higher in CSF compared to plasma.	[38]
Primary and secondary brain tumors	N = 7	cfDNA	*NF2, AKT1, BRAF, NRAS, KRAS, EGFR*	ddPCR, NGS	Mutations were detected in the CSF of 6 out of 7 patients while in the plasma of 4 out of the 7 patients	[39]
Medulloblastoma	N = 13	ctDNA	*GLI2, MYCN, PTCH1, BCOR, BLM, CTNNB1, KMT2D, MYC, PRDM6, PTEN, SUFU*	ddPCR	ctDNA was detected in the CSF of 76.9 % (10/13) of patients while in the plasma of only 1 of the patients with 2.2 % VAF.	[40]
NSCLC LM	N = 291 ALK + with LM = 30 ALK + without LM = 261	cfDNA	*ALK*	NGS	ALK rearrangement was identified in the CSF of 81.8 % of samples and in the plasma of 45.5 % of the sample. MAF in all CSF cfDNA was significantly higher than in the plasma cfDNA.	[41]
NSCLC LM	N = 92	cfDNA	*EGFR, MET, ALK, ERBB2, BRAF, KRAS, ROS1, TP53, CDKN2A, POM121L12, NTRK1, APC, CDK4, RB1, CTNNB1, SMAD4, FGF3, MYC, FGFR1, FGF4, CCND1, CDH18, OR4A15, PIK3R1, NAV3, FGF19, NOTCH1, PTEN, BRCA1, OR2T4, CDK6, BRINP3, KEAP1, AR*	NGS	Detection rate from CSF was significantly higher than that from plasma. MAFs was 43.64 % in CSF and 4.58 % in plasma. In total, 280 genomic alterations were identified in CSF samples, mapping to 60 genes, while 137 alterations were identified in plasma samples, mapping to 33 genes. Of these, 197 mutations were unique to CSF, and 54 were unique to plasma. The EGFR gene was the most frequently mutated with occurrences in 58.33 % of CSF samples and 44.44 % of plasma samples. A total of 121 CNVs were detected in CSF, with the majority (81.8 %) being specific to CSF, and only 18.2 % shared with plasma.	[42]
NSCLC with LM and BPM	N = 247	ctDNA	*EGFR, ALK, BRAF, KRAS, HER2, ROS1, MET, PIK3CA, STK11, TP53*	NGS	ctDNA was detected in the Plasma of 50 % patients, while in paired CSF of 83 % patients.	[43]

GBM = Glioblastoma, **BMBC** = brain metastasis from breast cancer, **BMLC** = brain metastasis from lung cancer, **NSCLC** = non-small cell lung cancer, **NGS** = next generation sequencing, **LM** = leptomeningeal metastasis, **BPM** = brain parenchymal metastasis, **cfDNA** = cell free DNA, **ctDNA** = circulating tumor DNA, **VAF** = variant allelic frequency, **MAF** = mutant allelic frequenncy

potential, and tissue biopsy remains the standard of care in clinical oncology practice [47–49]. The liquid biopsy efforts have primarily focused on peripheral venous blood samples, which generally contain low concentrations of tumor-derived biomarkers that are difficult to detect with standard assay platforms. Consequently, highly sensitive methods are required to detect such low levels, with most efforts currently focused on ctDNA. Additionally, methods to detect these rare molecules can introduce errors and result in false positives [47,50]. Taking biofluid closer to tumors can bypass these challenges and enrich biomarkers as evidenced in the literature [18,51,52].

Several surgical and endoscopic studies have reported higher levels of certain oncological biomarkers in the tumor-draining veins of solid malignancies compared to peripheral circulation [18,53,54] (Fig. 2). This suggests that the anatomy of the vascular system might influence the relative concentrations of various biomarkers in different vascular beds. Additionally, reports indicate that the properties and distributions of these markers in vascular beds proximal to a tumor may provide information on mutational status, metastatic potential, and immune evasion mechanisms [18,53–55]. This method is adapted from techniques long used in hormone assays and the diagnosis of neuroendocrine tumors, such as insulinomas [56,57]. Similarly, Farrelly et al. [58] demonstrated that free-to-total prostate-specific antigen percentage (fPSA%) was higher in internal iliac and deep internal iliac venous samples compared to peripheral venous (PV) samples in patients with prostate cancers. Wind et al. reported a higher quantity of CTCs in portal veins compared to PV in patients with primary colonic cancer. Some other studies also reported higher quantities of CTCs in tumor-proximal veins compared to the PV [18,59,60].

Similarly, Kawamura et al. [61] reported that the levels of exosome-encapsulated miR-4525, miR-451a, and miR-21 in Portal venous blood of Pancreatic ductal adenocarcinoma (PDAC) patients were significantly higher than those in PV of PDAC patients and healthy individuals.

Damascelli et al. [18] reported 3 cases and showed that selective

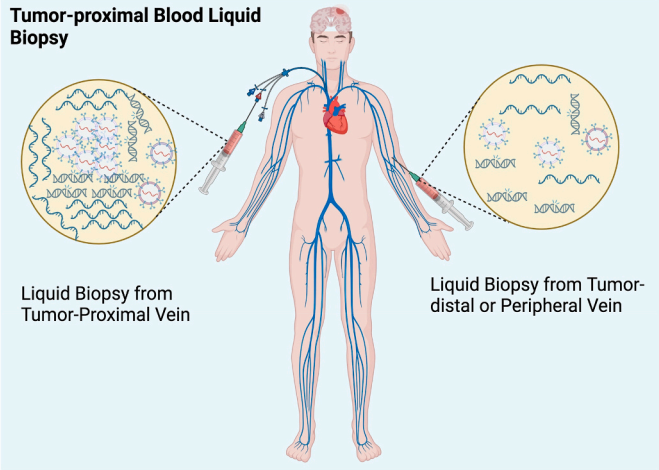

Fig. 2. Biomarkers are highly concentrated in tumor-proximal veins compared to peripheral veins. Blood is a commonly used fluid for liquid biopsy. As the biomarkers move away from the tumor and mix with the whole body's blood, the biomarkers become diluted. Blood in tumor-proximal veins provides more biomarkers compared to that of tumor-distal veins.

venous sampling can improve the sensitivity and specificity of liquid biopsy. For example, in the comparison of cfDNA, *Kirsten RAS oncogene homolog (KRAS)* mutation, and CTCs across different veins of a patient with pulmonary adenocarcinoma, the superior vena cava had a cfDNA concentration of 2.15 ng/μL, with a positive *KRAS* mutation and 3 CTCs detected. The inferior vena cava had a cfDNA concentration of 1.75 ng/μL, with no *KRAS* mutation or CTCs detected. Similarly, the peripheral vein had a cfDNA concentration of 1.55 ng/μL, with no *KRAS* mutation or CTCs detected [18].

There is no evidence in the literature to suggest that taking blood samples closer to brain tumors can enhance biomarker detection. However, previous studies provide evidence that taking blood closer to non-CNS tumors makes it easier to detect biomarkers than blood samples from peripheral veins [53–55]. Endovascular targeted blood sampling has been studied for many non-CNS malignancies, but currently, there is no evidence for CNS malignancies. We hypothesize that obtaining blood from the CNS tumor-draining vein can enrich the biomarkers and increase the sensitivity of diagnostic tools for detection.

5. Conclusion

CSF has great potential as a targeted liquid biopsy medium for brain malignancies. CSF provides a more direct and enriched source of tumor-derived biomarkers than peripheral blood. Its unique proximity to the central nervous system allows for the detection of higher concentrations of circulating tumor DNA (ctDNA), microRNA (miRNA), proteins, and extracellular vesicles (EVs) that are often undetectable in systemic circulation due to the blood-brain barrier and other physiological challenges. This advantage makes CSF a superior biofluid for improving the sensitivity and accuracy of diagnostic tools in brain tumor patients. While the current standard for liquid biopsy in oncology primarily relies on peripheral blood, evidence suggests that targeted sampling closer to the tumor site, such as from CSF or CNS tumor-draining veins, could significantly enhance biomarker detection and diagnostic outcomes. Although more research is needed to validate this approach for CNS malignancies, promising results from non-CNS tumors, indicate a potential shift in how we approach liquid biopsy for brain cancers. Future studies should focus on further exploring and refining these methods to fully realize the benefits of CSF-based liquid biopsy, potentially leading to more precise and timely diagnosis and monitoring of brain malignancies.

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Financial relationships

All authors have declared that they have no financial relationships at present or within the previous three years with any organizations that might have an interest in the submitted work.

Other relationships

All authors have declared that there are no other relationships or activities that could appear to have influenced the submitted work.

Ethical Approval/Patient Consent

Ethical Approval/Patient Consent was not required.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Gene Symbol	Full Form
EGFR	Epidermal Growth Factor Receptor
KIT	KIT Proto-Oncogene, Receptor Tyrosine Kinase
PIK3CA	Phosphatidylinositol-4,5-Bisphosphate 3-Kinase Catalytic Subunit Alpha
TP53	Tumor Protein P53
SMAD4	SMAD Family Member 4
ATM	Ataxia Telangiectasia Mutated
SMARCB1	SWI/SNF Related, Matrix Associated, Actin Dependent Regulator of Chromatin, Subfamily B Member 1
PTEN	Phosphatase and Tensin Homolog
FLT3	Fms Related Receptor Tyrosine Kinase 3
GNAS	GNAS (Guanine Nucleotide binding protein, Alpha Stimulating activity polypeptide) Complex Locus
STK11	Serine/Threonine Kinase 11
MET	MET (Mesenchymal Epithelial Transition) Proto-Oncogene, Receptor Tyrosine Kinase
CTNNB1	Catenin Beta 1
APC	Adenomatous Polyposis Coli
FBXW7	F-Box and WD Repeat Domain Containing 7
ERBB4	Erb-B2 Receptor Tyrosine Kinase 4
KDR	Kinase Insert Domain Receptor
ERT	Estrogen Receptor 1
IDH1	Isocitrate Dehydrogenase 1
CDKN2A/B	Cyclin Dependent Kinase Inhibitor 2A/B
ATRX	ATRX Chromatin Remodeler, Alpha Thalassemia/Mental Retardation Syndrome X-Linked
CIC	Capicua Transcriptional Repressor
NF1	Neurofibromin 1
PIK3R1	Phosphoinositide-3-Kinase Regulatory Subunit 1
CDK4	Cyclin Dependent Kinase 4
RB1	Retinoblastoma 1
PDGFRA	Platelet Derived Growth Factor Receptor Alpha
SOX2	SRY-Box Transcription Factor 2
NOTCH1	Notch Receptor 1
MDM2	MDM2 Proto-Oncogene
SETD2	SET Domain Containing 2
PTPN11	Protein Tyrosine Phosphatase, Non-Receptor Type 11
FUBP1	Far Upstream Element Binding Protein 1
KMT2D	Lysine Methyltransferase 2D
FAT1	FAT Atypical Cadherin 1
H3F3A	H3 Histone, Family 3A
PPM1D	Protein Phosphatase, Mg2+/Mn2+ Dependent 1D
HIST1H3B	Histone Cluster 1H3 Family Member B
ACVR1	Activin A Receptor Type 1
FGFR1	Fibroblast Growth Factor Receptor 1
BCOR	BCL6 Corepressor

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Gene Symbol	Full Form
BRAF	B-Raf Proto-Oncogene
FGFR3	Fibroblast Growth Factor Receptor 3
RGPD3	RANBP2-Like and GRIP Domain Containing 3
BRCA2	Breast Cancer gene 2, DNA Repair Associated
MLH1	MutL Homolog 1
MSH6	MutS Homolog 6
NF2	Neurofibromin 2
AKT1	AKT Serine/Threonine Kinase 1
NRAS	NRAS Proto-Oncogene, GTPase
GLI2	GLI Family Zinc Finger 2
MYCN	MYCN Proto-Oncogene, BHLH Transcription Factor
PTCH1	Patched 1
SUFU	Suppressor Of Fused Homolog Negative Regulator Of Hedgehog Signaling
ROS1	ROS Proto-Oncogene 1, Receptor Tyrosine Kinase
POM121L12	POM121 Transmembrane Nucleoporin Like 12
NTRK1	Neurotrophic Receptor Tyrosine Kinase 1
FGF3	Fibroblast Growth Factor 3
FGF4	Fibroblast Growth Factor 4
CCND1	Cyclin D1
CDH18	Cadherin 18
OR4A15	Olfactory Receptor Family 4 Subfamily A Member 15
NAV3	Neuron Navigator 3
FGF19	Fibroblast Growth Factor 19
BRCA1	Breast Cancer gene 1, DNA Repair Associated
OR2T4	Olfactory Receptor Family 2 Subfamily T Member 4
CDK6	Cyclin Dependent Kinase 6
BRINP3	BMP/Retinoic Acid Inducible Neural-Specific Protein 3
KEAP1	Kelch Like ECH Associated Protein 1
AR	Androgen Receptor

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