JEIMP
The Journal of European Internal Medicine Professionals

Review

Exploring The Role of Circulating Biomarkers in Glioblastoma Multiforme: Bridging The Gap Between Laboratory and Clinic

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J Eur Int Prof. Year; 2025, Volume: 3, Issue: 2
Submitted at: 02.02.2025 Accepted at: 16.03.2025 Published at: 25.03.2025

doi 10.5281/zenodo.15061495

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Abstract

Glioblastoma multiforme is the most prevalent and aggressive primary malignant brain tumor in adults, characterized by significant intratumoral heterogeneity and resistance to conventional therapies. Despite improvements in surgical resection, radiotherapy, and chemotherapy with temozolomide, GBM remains incurable, with a median survival of 10–15 months. Current diagnostic modalities include magnetic resonance imaging and tissue biopsies, face early detection, real-time monitoring, and comprehensive tumor profiling limitations. These challenges underscore the urgent need for minimally invasive, highly specific, and sensitive diagnostic tools. Liquid biopsy has emerged as a promising alternative, enabling the detection of circulating biomarkers, including circulating tumor cells, cell-free nucleic acids, extracellular vesicles, and proteins from biofluids such as blood and cerebrospinal fluid. These biomarkers offer insights into tumor heterogeneity, therapeutic resistance, and progression while facilitating dynamic treatment response monitoring. This review explores the potential of circulating biomarkers in revolutionizing GBM diagnosis and management, focusing on their molecular characteristics, clinical utility, and limitations. By integrating these innovative approaches into clinical practice, liquid biopsy has the potential to significantly improve patient outcomes, heralding a new era in the diagnosis, prognosis, and therapeutic monitoring of GBM.

Keywords: Glioblastoma Multiforme, Circulating Biomarkers, Liquid Biopsy

INTRODUCTION

Glioblastoma multiforme (GBM) stands as the most common primary malignant brain tumor observed in adults, characterized by the highest mortality rate and notable aggressiveness. These tumors, known for their high mitotic activity and susceptibility to necrosis (1), account for approximately 14.5% of all Central Nervous System (CNS) tumors and nearly half of (48.6%) all malignant CNS tumors (2). Commonly located in the supratentorial region of the brain, GBMs are less frequently observed in the cerebellum, brainstem, and ventricles (3). GBMs arise as a result of uncontrolled growth of cells known as glia/neuroglia, which participate in neuronal activity, protect and support neurons, and cause symptoms such as headache, weakness, memory problems, personality changes, vision and speech difficulties, seizures, and paralysis arise due to the tumors compression of neighboring cells (1, 4). The majority of GBM patients, approximately 70%, face a poor prognosis, with a median survival ranging from 10 to 15 months and a 5-year survival rate of roughly 7% (5).

The etiology of GBM results from complex interactions between environmental factors and genetic predispositions, but the exact mechanisms of this relationship remain unclear (6). The carcinogenetic causes and mechanisms underlying the disease are not fully known. Although exposure to ionizing radiation is considered a risk factor for GBM, no definitive association has been found between GBM and environmental factors like tobacco use, electromagnetic fields, head injuries, and exposure to pesticides (4).

The conventional treatment approach for GBM currently involves surgical tumor resection, radiotherapy (RT), and chemotherapy (ChT) with the drug temozolomide

(TMZ) (7). The first step in treatment is to surgically remove as much of the tumor as possible, as this method is linked to longer progression-free and overall survival (OS) (8). Due to the diffuse infiltrative nature of GBMs, surgical resection is typically not curative. Instead, it aims to enhance survival, alleviate neurological symptoms, and improve patients' capacity to undergo postoperative treatments. The main features that complicate the treatment of GBM are the tumor's genetic, epigenetic, morphological, and histopathological heterogeneity. This type of heterogeneity encourages relapses that are resistant to treatment over time by causing the tumor to adapt to treatment and become resistant (9). Since resection is not a definitive therapeutic approach, RT and ChT are additionally administered to patients. While the OS in patients receiving RT alone is 12.1 months and the two-year survival rate is 10.4%, the OS is extended to 14.6 months, and the two-year survival rate is increased up to 26.5% with TMZ treatment in addition to RT (10). Despite this increase in survival with concurrent treatment with RT and TMZ, tumor progression and recurrence are typically inevitable due to the development of resistance to TMZ and hemotologic toxic effects (8). The challenges in treating GBM persist due to incomplete tumor resection, significant intratumoral heterogeneity, difficulty crossing the blood-brain barrier (BBB), and the immunosuppressive tumor microenvironment. The infiltrative nature of GBM makes it nearly impossible to achieve complete cellular-level resection. Additionally, hypoxic regions within the tumor create perivascular niches that support Glioblastoma-initiating cells (GICs). These self-renewing GICs contribute to the formation of more aggressive recurrent tumors that are resistant to both RT and ChT (11). Despite all these treatment options, GBM is still not completely curable, and recurrence rates after treatment are pretty high (2). Therefore, early diagnosis of these malignant tumors is crucial.

Considering today's technological advances, no screening tool or test has been developed to identify GBM before clinical symptoms emerge (2). GBM is diagnosed by computed tomography (CT) or magnetic resonance imaging (MRI) followed by biopsy for confirmation. However, the most important method for radiologically diagnosing GBM is MRI, as it outperforms CT with superior anatomical resolution, offers better delineation of GBM characteristics, and the ability to perform more advanced analyses such as brain tumor spectroscopy (5). There are also many limitations to the use of MRI for diagnosis. Due to the resolution limit of MRI, lesions as small as 2-3mm in size, particularly in the early stages, may be overlooked, which affects diagnostic accuracy and makes it difficult to detect early-stage tumors. Furthermore, it is not always possible to clearly distinguish GBM from other tumors and CNS diseases (12). GBM may therefore be mistaken for other brain tumors such as low-grade gliomas, brain metastases, or primary CNS lymphoma, as well as with nonneoplastic disorders like brain abscesses, demyelinating diseases, and hemorrhagic transformation of ischemic strokes (5). In some cases, MRI scans are also used to obtain prognostic information after treatment. In this case, lesions caused by tumor progression cannot be reliably distinguished from pseudoprogression (PsP). These treatment-related changes mimic tumor progression and could resolve independently over time. PsP occurs in 10-30% of GBM patients undergoing initial MRI scanning, typically within the first 12 weeks of treatment (13). In cases where surgery is not deemed appropriate, tissue analysis through stereotactic biopsies can help differentiate PsP from true tumor recurrence, but this further exposes patients to nonnegligible surgical risks.

Intraoperatively resected tumor tissue is needed for definitive diagnosis by histopathological examination. When tumor resection is not possible, or metastatic GBM is suspected, a fine needle aspiration biopsy is carried out from accessible areas (1). The intratumoral heterogeneity of GBM is an important factor in treatment compliance and resistance to treatment. In addition to the difficulty of obtaining tissue biopsy (TB) specimens and the serious potential complications of the procedures, the collected specimens may not fully represent the entire tumor due to intratumoral heterogeneity. Therefore, due to their invasive nature and limited sampling capacity, whole TBs cannot assess tumor activity in real-time (5). The risks involved in GBM TBs often prevent repeat sampling during tumor progression, restricting opportunities to monitor treatment response and identify therapeutic resistance at an early stage. The challenges posed by MRI and TBs emphasize the urgent and unmet clinical need for innovative, alternative, and complementary diagnostic techniques to improve the diagnosis, treatment, and follow-up of GBM patients (5). These challenges have led to the search for less invasive and more accurate methods in the diagnosis and treatment process. In this context, against the limitations of conventional diagnostic methods, circulating biomarkers have great potential for detecting and monitoring tumors in their early stages. The use of circulating tumor cells (CTCs), circulating cell-free nucleic acids (ccfNAs), extracellular vesicles (EVs), and circulating proteins (CP) are increasingly being investigated for monitoring patients' response to treatment with less invasive methods (14).

Biomarkers serve a vital role in the molecular profiling of the tumor, enabling more accurate prognostic predictions for personalized treatment strategies and early detection of treatment-resistant relapses. The aim of this review is to examine the potential of circulating biomarkers for diagnosing and monitoring GBM, to discuss new approaches that may provide alternatives to current methods, and to provide a perspective on how

these biomarkers can be used in clinical practice.

METHODS

The literature search was conducted in scientific databases including PubMed, Web of Science, and Scopus using keywords such as "glioblastoma multiforme", "circulating biomarkers", "liquid biopsy", "circulating tumor cells (CTCs)", "cell-free nucleic acids (cfNAs)", "extracellular vesicles (EVs)" and "circulating proteins (CPs)" to ensure a comprehensive and systematic review. We included research articles published in leading high-impact, peer-reviewed journals with strong experimental designs, in vitro and in vivo findings supported by clinical outcomes, and review articles published in the last 5 years discussing the advantages of liquid biopsy and circulating biomarkers and advances in their role in GBM. To ensure a balanced representation of the latest scientific advances, studies with large sample sizes, robust statistical analyses, and direct relevance to circulating biomarkers in GBM were prioritized for their potential importance. On the other hand, we excluded non-English language articles, conference abstracts, and articles that focused solely on preclinical models without human data.

LIQUID BIOPSY and CIRCULATING BIOMARKERS: PIONEERING A NEW ERA in DIAGNOSTICS and MONITORING of GBM

Tumor development is an extremely comprehensive and complex process, limited to physiological and metabolic changes and leading to various molecular and biochemical alterations (6). In order to manage this process effectively, the discovery of potential biomarkers is of great importance. Biomarkers are biological indicators that allow us to assess normal biological functions, pathological states, and pharmacological responses to therapeutic interventions with high sensitivity and specificity (15). In recent years, several diagnostic and prognostic biomarkers of aggressive glioblastomas have been identified and have contributed significantly to the accuracy of diagnosis and efficacy of treatment (6,16). Especially in GBMs, liquid biopsy (LB) is pioneering a new era (17), facilitating early diagnosis of GBM by recognizing neoplastic transformations, managing tumor progression, and optimizing patient follow-up by monitoring response to treatment (18). LB, as opposed to TB, is a minimally invasive diagnostic method with advantages, such as easy applicability, speed, cost-effectiveness, reproducibility, high sensitivity, real-time monitoring, and capacity to represent tumor heterogeneity (19,20). It provides detailed information about tumor evolution over time by detecting and quantifying tumoral contents released by tumors into biological fluids such as blood, cerebrospinal fluid (CSF), saliva, urine, and cyst fluid (17,19).

Different biological fluids offer distinct advantages and limitations in detecting circulating biomarkers for GBM, influencing their diagnostic accuracy and clinical applicability. Peripheral blood (PB) is the most investigated biological material for the determination of circulating biomarkers in GBM due to its advantages in accessibility, ease of collection, minimal invasiveness, and dynamically reflecting tumor progression. However, its diagnostic utility for GBM is limited by the selective permeability of the BBB, which restricts the release of tumor-derived molecules into circulation. As a result, blood-based biomarkers often exhibit lower sensitivity and specificity compared to other biological fluids, particularly in early-stage disease detection. In addition, CSF is also used as an ideal source of LB due to its potential for direct contact with the central nervous system and tumor microenvironment, making it a highly enriched source of GBM-related biomarkers (17). Biomarkers such as ccfDNA, EVs, and CPs are more abundant in CSF than PB, which is thought to lead to improved biomarker detection rates. However, CSF collection via lumbar puncture or ventricular catheterization is invasive limiting its routine use in clinical practice. Moreover, CSF sampling is not always feasible, particularly in patients with increased intracranial pressure or where tumor location restricts safe access. Urine, a completely non-invasive biological fluid, has recently emerged as a potential alternative for biomarker detection. EVs and microRNAs can be detected in urine samples of GBM patients. However, due to the low concentration of brain-derived molecules in urine, its diagnostic utility remains highly uncertain. Additionally, renal clearance and metabolic degradation further complicate biomarker stability, making urine-based GBM diagnostics less reliable compared to PB and CSF. Saliva has also been investigated as a potential biological fluid for detecting brain tumor biomarkers. Saliva collection is non-invasive and offers advantages such as ease of repeated sampling. EVs and ccfRNAs can also be detected in saliva, potentially reflecting tumor biology. However, the reliability of saliva-based biomarker detection for GBM remains highly questionable, as saliva primarily contains molecules derived from local oral and salivary gland tissues. Additionally, the low concentration of GBM-related biomarkers and potential contamination from other systemic factors limit its applicability in routine clinical settings. Cyst fluid collected from cystic GBM lesions presents a unique biological fluid with potentially high concentrations of tumor-derived biomarkers. Cystic components of GBM contain ccfDNA, EVs, and CPs, which may offer insights into tumor heterogeneity and progression. Cyst fluid may provide a more direct representation of tumor biology compared to PB. However, the clinical application of cyst fluid as a biomarker source is limited by the infrequent occurrence of cystic GBMs and the invasive

nature of fluid collection, which typically requires stereotactic aspiration. Given these differences, CSF remains the most promising biological fluid for GBM LB, while plasma offers a more practical alternative for longitudinal monitoring. However, due to their non-invasiveness, urine, and saliva-based biomarkers require further validation before they can be integrated into routine clinical practice. Despite its high biomarker content, cyst fluid only applies to a subset of GBM cases. Future research should focus on standardizing biological fluids selection criteria and optimizing detection methodologies to enhance the clinical applicability of LB in GBM.

Two types of biomarkers can be found in LB: Tumor-derived markers originating from the tumor itself and tumor-related markers stemming from the body's response to the tumor. Although tumor-associated biomarker discovery is much more complex, their importance in diagnosis, treatment, and prognosis in GBM is much greater (21). In conclusion, LB is a valuable complementary tool to current clinical strategies in the diagnosis and treatment follow-up processes of GBM, which is gaining more and more importance. CTCs, ccfNAs, EVs, and CPs are tumor-derived biomolecules, and studies on their importance in GBM have accelerated in recent years (5,19,22).

CIRCULATING BIOMARKERS in GBM

Circulating Tumor Cells (CTCs) and Their Association with GBM

The presence of CTCs was first identified by Australian researchers in 1869 (14). CTCs have long been known for their role in clinical applications, including cancer detection, genetic profiling, tumor progression tracking, and tailoring personalized therapies. CTCs are tumor-derived cells released into the bloodstream by primary tumors during their formation or growth, which can spread to distant sites and eventually metastasize (23). These cells demonstrate the metastatic potential of epithelial tumor cells (24). Although CTCs are implicated in the development of metastasis, the exact mechanisms involved in this process are intricate and have not yet been clarified (25). The transition of CTCs from epithelial to mesenchymal phenotypes results in the loss of cell-to-cell adhesion, the acquisition of less differentiated mesenchymal characteristics, enhanced migratory potential of cells, and intravasation into circulation. (5, 14). This process led to CTCs becoming an attractive target for tumor biomarker research aimed at early diagnosis in the 1960s and 1970s (21).

While CTCs offer great potential in diagnosing GBM, their integration into clinical practice faces several challenges. The selectively permeable nature of the BBB, the short 24-hour half-life of CTCs, the physical barrier created by endothelial cells in the blood-vessel barrier for tumor cells to enter the circulation, the hemodynamic forces experienced in the circulation, the lack of growth factors and extracellular matrix support, the attack of host immune system cells that suppress tumor migration, and restriction of the mobility of cells in circulation and their capacity for metastasis as a result of interaction with fibrin networks or platelets make the CTCs intravasation into the circulation even more difficult. Even if millions of CTCs are released into the bloodstream from tumors, the concentration of them remains at ~1-10 cells per 10 mL of blood, or 1 in 109 cells. This explains why CTCs are technically difficult to detect with high specificity and sensitivity (21).

Nevertheless, the potential for investigating CTCs in clinical trials is increasing daily. Recent studies have confirmed the presence of CTCs with glial features in the PB of GBM patients, and it has been shown that the genomic content of these cells accurately represents the tumor of origin (26). CTCs are as common as 75% in GBM (27). These findings place CTCs in a promising position not only as an innovative tool in GBM diagnosis and follow-up but also as a promising alternative to conventional TB. The potential of CTCs to offer the advantages of biopsy to a broad patient population in a minimally invasive approach further increases the clinical value of LB applications (28).

A study by Müller et al. showed that CTCs were present in the blood of 29 of 141 (20.6%) GBM patients by immunochemical analysis using immunostaining of mononuclear cells enriched with antibodies against glial fibrillary acidic protein (GFAP). In addition, the presence of CTCs in PB was evaluated prior to and following surgical resection. CTCs were found in both pre- and post-surgical samples in 13.4% of patients, only in post-surgical samples in 7.5%, and only in pre-surgical samples in 6% (27).

CTCs may also be helpful in monitoring the response to treatment in GBM patients. In a study conducted by Gao et al. on 31 patients with seven different pathologic types of primary glioma at WHO stages II, III, and IV, the incidence and number of CTCs in the PB of patients preoperatively and 1 week postoperatively were determined. CTCs were observed in the blood of 24 of 31 (77.4%) patients with primary glioma and 9 of 11 (81.8%) patients with GBM. The researchers reported that the CTC counts of postoperative patients decreased significantly compared to pre-treatment levels. When postoperative CTC counts were analyzed, it was found that CTC counts decreased in 19 of 24 (79.2%) patients with primary glioma and 7 of 9 (77.8%) patients with GBM who had CTCs detected in their blood before the operation. They concluded that detecting of CTCs may contribute to differentiating of radiation necrosis from actual tumor progression (29). In another study supporting these findings, CTCs were

identified in 72% of patients with GBM, and this rate decreased to 8% after RT. The detection of CTCs was performed with a method based on telomerase activity, taking advantage of the high sensitivity and specificity provided by the high telomerase expression seen in over 90% of solid tumors despite the absence of telomerase expression in normal cells. The results of the study show that telomerase activity-based strategies have significant potential for evaluating treatment response and monitoring disease recurrence in patients receiving RT (30). In a retrospective analysis of 22 patients who had tumor resection followed by RT and subsequently developed new mass lesions on MRI, the number of CTCs was significantly higher in the tumor recurrence group compared to the tumor necrosis group (31). Sullivan et al. reported the presence of CTCs in 13 PB samples from 33 (39%) GBM patients at different stages of treatment (32).

The reported detection rates of CTCs in GBM patients vary significantly in the literature, ranging from 20% to 75%. This significant variability across studies can be attributed to differences in detection methodologies, patient selection criteria, cohort characteristics, and blood sample collection timing. Immunostaining techniques like GFAP-based enrichment yield lower detection rates compared to PCR-based approaches and microfluidic platforms, which offer higher sensitivity but may also capture non-tumor-derived circulating cells. Additionally, the classification of study participants, whether newly diagnosed or recurrent GBM patients, can substantially influence CTC detection rates. Beyond methodological differences, patient-specific factors such as tumor stage, variations in BBB integrity, and systemic inflammation can also affect the release of CTCs into circulation, thereby impacting their detectability. Similarly, the timing of biological liquid sampling, whether collected pre- or post-treatment, further contributes to the broad range of reported detection rates.

CTCs can circulate as single cells or homotypic/heterotypic clusters showing higher metastatic potential (5, 7). It has been reported that CTCs form clusters with white blood cells (WBCs), and even the presence of CTC-WBC clusters indicates poor prognosis in some tumors, such as hepatocellular carcinoma (33,34). Szczerba et al. concluded that CTC-neutrophil clusters injected into tumor-free mice accelerated tumor formation, increased metastatic potential, and shortened OS as compared with single CTCs (34).

In GBM management, CTCs should provide concrete evidence to improve the efficacy of therapeutic strategies and have a meaningful impact on the disease course. Currently, the presence of CTCs is considered a potential tool detecting prognostically important genetic biomarkers for GBM, such as isocitrate dehydrogenase (IDH) mutations, although CTCs alone are not of sufficient clinical value. However, including CTCs in a broader panel of biomarkers for GBM patients could contribute significantly to diagnostic accuracy and clinical utility.

Circulating Cell-Free Nucleic Acids (ccfNAs) and Their Association with GBM

Cells can release their nucleic acids into circulation. The presence of ccfNAs was first detected in 1948 by Mandel and Metais in the PB of healthy individuals and patients diagnosed with various metabolic and/or oncological diseases (35). Circulating cell-free DNA (ccfDNA) consists of small DNA fragments, approximately 180-200bp in length, released under physiological and pathological conditions and thought to originate mainly from apoptotic cells (36). The ccfDNA originating from normal cells is usually derived from genomic DNA released during apoptosis or inflammation, and its concentration in the blood is low as it is rapidly removed by phagocytes (37). When phagocytic removal is insufficient in cancer, DNA fragments released from apoptotic and/or necrotic cells of tumor origin accumulate in the circulation. Tumor cells can similarly release different classes of RNAs into the bloodstream, such as protein-coding mRNA, small noncoding microRNAs (miRNAs) of approximately 21-24 nucleotides, and long noncoding RNAs of 200 nucleotides or more. In PB and CSF samples of the GBM patients, circulating cell-free RNAs (ccfRNAs) have been demonstrated, emphasizing their potential as biomarkers for prognosis, diagnosis, and monitoring treatment responses (38).

In 1977, Leon et al. reported higher amounts of ccfDNA in cancer patients than in noncancerous individuals (39). Stroun et al. showed that tumor-associated genetic alterations were found in ccfDNA in cancer patients, and subsequent studies confirmed that neoplastic genomic alterations such as mutations in oncogenes or tumor suppressor genes, microsatellite instability and epigenetic variations can be detected in tumor-derived ccfDNA fragments known as circulating tumor DNA (ctDNA) (40). The demonstration that ccfDNA carries the same molecular information as biopsy samples obtained from tumor tissue has paved the way for ctDNA as a potential biomarker for diagnosing and monitoring cancers (41).

Several studies have identified the presence of ctDNA in some cases with primary CNS tumors, including astrocytoma and oligoastrocytoma. GBM is distinguished from other neoplasms by the low ctDNA concentrations and positive index found in the serum of patients. The proportion of ctDNA among all ccfDNA correlates with tumor burden in advanced-stage solid tumors. ctDNA provides a dynamic reflection of tumor progression and contributes to understanding the mechanism underlying gene mutations and drug resistance in primary tumors (42). In addition, ctDNAs reflect the

molecular profile of tumors, including information on targeted mutations in patients with CNS tumors and drug resistance mechanisms in targeted therapy. Through ctDNA analysis, tumor progression and drug resistance mutations can be identified early (6). This approach has been successfully used to detect specific mutations in adult and pediatric patients with brain tumors. Mutations in genes such as O-6-methylguanine-DNA methyltransferase (MGMT) promoter in astrocytic and oligodendroglial tumors (43, 44), death-associated protein kinase (DAPK) in GBMs (45), phosphatase and tensin homolog (PTEN) in astrocytic tumors and GBMs (43, 46), and epidermal growth factor (EGFR) and IDH (46) in gliomas are examples of ctDNA markers (21). Although TB for histological diagnosis and to obtain information on tumor biomarkers is still valid today, the potential of ctDNA as a biomarker leads to promising approaches in the clinic (47).

ctDNA carries tumor-specific mutations that reflect the mutational characteristics of the primary tumor. Therefore it has significant potential in clinical applications for noninvasive tumor tissue sampling (7). To assess whether ctDNA can facilitate genomic interrogation, Piccioni et al. clinically analyzed data from 419 primary brain tumor patients with a next-generation sequencing panel. The ctDNA mutation rate per patient stratified by histologic subtype was 55% in 222 GBM cases. The researchers report that a biopsy-free option, thanks to ctDNAs, shows promise and could provide a pathway for further advances in genomically matched clinical trials (48). In a study by Lavon et al. evaluating the potential of ccfNA as a noninvasive tool for identifying genetic/epigenetic changes in high-grade astrocytomas and oligodendrogliomas during the disease, loss of heterozygosity (LOH) and/or methylation on chromosomes 1p, 19q and 10q that could identify DNA as tumor-specific was detected in 80.5% of astrocytomas and all oligodendrogliomas. The detection rates of these biomarkers in serum were 51% and 55%, respectively, and the specificity was reported to be around 100%. According to these data, ccfDNA in glial tumors was reported to be informative for both LOH and methylation analysis throughout the progression of the disease (43). In a similar study in 2012, Boisselier et al. first attempted to detect ctDNA-based IDH mutations in glioma and found the mutations in 15 of 25 patients (60%) with mutated tumors. In contrast no mutations were detected in 14 patients with wild-type tumors. Sensitivity increased proportionally with tumor volume, and specificity was 100% (49). In a study by Wang et al., relevant tumor tissues from 89 glioma patients were analyzed for MGMT promoter methylation. It was reported that detection of MGMT promoter methylation in CSF samples (65.0%) showed higher sensitivity compared to serum samples (37.3%) (50).

Studies have shown that ctDNA detection rates are greater in CSF than in plasma and serum. One possible reason for this is that the BBB, even if partially impaired, limits the entry of ctDNA from the primary brain tumor into the bloodstream (51). Despite the promising results, using ctDNA as a biomarker for GBM, in particular, remains challenging. Firstly, ctDNA constitutes 0.1% to 5% of the total ccfDNA, varying according to tumor type, grade, and burden (14). Furthermore, gliomas were among the tumor types with the lowest level of detectable ctDNA. Secondly, ctDNA has a short half-life of less than two hours, requiring rapid processing after sample collection. Thirdly, even if detectable, ctDNA levels in blood are very low in cancer patients, necessitating highly sensitive techniques for its identification and differentiation from normal ccfDNA (52).

Upregulation of miR-21 in plasma (53) and tissues (54) of GBM patients has been reported and shown to be associated with lower OS and tumor grading (55). Wang et al. analyzed plasma from ten GBM patients before and after treatment and identified two miRNAs, miR-128 and miR-342-3p, that were down-regulated in patients compared to controls. miR-128 and miR-342-3p levels were associated with glioma grades and increased following surgery and chemoradiation, indicating their potential as biomarkers for tumor grading and treatment response assessment (56).

To aid molecular diagnosis in GBM, monitor tumor response, identify early recurrence, and follow glioma clonal evolution, although ccfNAs may be helpful as a minimally invasive tool to characterize recurrent tumors and lead to targeted therapies molecularly, there is insufficient evidence for the use of ccfNAs as a biomarker in GBM in routine clinical practice and large prospective studies are still needed to confirm how reliably ccfNAs can reflect the mutational character of GBM, especially when using comprehensive genomic technologies (57).

Circulating Extracellular Vesicles (cEVs) and Their Association with GBM

EVs are small vesicles surrounded by a membrane-bound bilayer lipid membrane secreted into the extracellular space by both healthy and tumor cells under physiological or pathological conditions (6). Structurally composed of various cellular components such as proteins, lipids, and nucleic acids, EVs are heterogeneous in terms of their size, origin, nature, and quantity of molecular content and biological activity and are categorized according to these characteristics. The most widely studied categories of EVs are exosomes, ranging in size from 50 to 150nm, and microvesicles (MVs), ranging from 50 to 1000nm (58). Exosomes are intraluminal vesicles that form into the endosomal membrane during the maturation of multivesicular endosomes (MVEs). The fusion of MVEs with the cell membrane results in the

release of exosomes into the extracellular space (5). MVs are vesicles formed by direct budding of the outer cell membrane (58). Other subclasses of EVs include apoptotic bodies and oncosomes, which are formed nonviably due to apoptosis (59).

EVs mediate intercellular communication (21) and modulate recipient cells' molecular functions by releasing diverse biological factors (6). Therefore, tumor cells secrete exosomes carrying tumor-specific biomarkers, enabling the identification of primary tumor properties. EVs secreted by neoplastic cells can induce the response of neighboring stromal cells with their molecular content, induce direct EV-target cell surface contact by affecting the corresponding membrane-associated receptors, and even alter the program of recipient cells by transferring relevant functions to target cells (60). Pioneering studies have shown that cEVs are critical in generating resistance in GBM. Tumor cells use cEVs to regulate processes such as modulating the tumor microenvironment to promote tumor growth, proliferation, angiogenesis, immune tolerance, drug resistance, modification of tumor metabolism, metastasis, invasion, and avoidance of cell death (61). Exosomes can shape tumor progression, suppress antitumor immunity by promoting angiogenic activity, and accelerate metastatic tumor growth. With these functions, they may contribute to tumor progression. Studies have shown that exosome components largely depend on their initial host cells, suggesting that exosomes carry or mimic the information of their parent cells. Exosomes may represent useful cancer diagnostic biomarkers (62).

Under physiological conditions, tumor cells produce cEVs at a higher rate than normal cells (24). Unlike ctDNAs released from apoptotic cells, cEVs originate from living cancer cells and retain their content from enzymatic degradation (63). cEVs derived from tumor cells are known to be associated with prognosis in many cancers. Mutations of KRAS, EGFR, BRAF, and TP53 genes in DNA in tumor-derived exosomes have been identified in pancreatic, non-small cell lung carcinoma, melanoma, and colorectal cancer, respectively (64). Patient-derived cell lines and cEVs also contain brain tumor markers such as HER2, EGFR, and mutant IDH (65, 66).

The ability to circulate in various body fluids like CSF, urine, and plasma gives cEVs the benefit of being a noninvasive testing alternative. In addition, cEVs are a stable tool for genomic testing as their lipid bilayers protect biomacromolecules such as RNA, DNA, and proteins from enzymatic activity (67). Due to the increased secretion of cEVs by neoplastic cells, they may serve as a rich source of information about GBM's heterogeneous biodiversity, tumor condition, and disease progression (17).

GBM cells have been observed to secrete cEVs that interact with endothelial cells to induce angiogenesis and stimulate tumor cell growth via an autocrine mechanism. Skog et al. provided evidence that cEVs can be obtained from the serum of brain tumor patients and that specific genetic alterations in the EGFR gene can be detected in these cEVs (68). In addition, studies in the serum of GBM patients revealed that different RNA expression profiles can be detected in the cEVs of tumor patients compared to the control group (69). Osti et al. found that plasma concentrations of cEVs were increased in patients with GBM compared to healthy individuals, and this was related with recurrence after tumor resection. In the same study, in order to examine how the cEV proteome is affected by GBM, protein profiles of plasma cEVs obtained from matched GBM patients before and after surgery were extracted, and the expression differences of 102 proteins in the pre- and postoperative groups were shown. It has been suggested that cEVs may be a valuable biomarker to distinguish patients with GBM from other brain injury-related diseases and be useful in early diagnosis (70). Some studies suggest that TMZ treatment may affect cEV release and potentially lead to drug resistance. Analyzing the molecular profile of cEVs may be useful in monitoring the efficacy of TMZ treatment (14). All these features make cEVs an important study area for developing new therapeutic alternatives in glioma (71).

All these clinical data suggest that cEVs may have a potential role in the diagnosis, follow-up, and prognosis of GBM. However, there are also some current limitations. One of the biggest challenges in this field is the lack of standard protocols for cEV enrichment and characterization and the difficulty of cEV research in achieving consistency on a specific standard. This lack of standardized protocols for the isolation, analysis, and reporting of cEVs reduces the comparability of results obtained in different laboratories or studies and leads to complexity (21). In addition, it is still unclear which biosolids are the most appropriate or sufficient source of GBM-derived cEVs. Especially in GBM patients, the permeability of the BBB for cEVs is well known, so plasma, CSF, urine, or saliva may be a more suitable option for cEV fluid biopsy (21). In addition, a limited number of studies demonstrate the isolation and characterization of cEVs from a large number of complex specimens. Studies with larger cohorts are needed to clinically validate the cEVs potential role and see whether they can distinguish GBM from other brain tumors (7).

Circulating Proteins (CPs) and Their Association with GBM

CPs detectable in serum have been widely studied for their potential as biomarkers for many types of cancer. However, since no GBM-specific protein has been

identified so far, studies on detecting changes in the levels of some proteins released into the circulation, specifically from GBM tumor cells, have gained momentum (26). Finding a protein with biomarker potential is very important in diagnosing and monitoring response to treatment, especially in aggressive tumors such as GBM. As a result of studies conducted in this context, proteins such as immunosuppressive acidic protein (IAP), alpha-1 acid glycoprotein (AGP), alpha-1 antitrypsin (AAT), fibronectin and thrombomodulin-1 (TM-1) stand out among the protein biomarkers detected for the first time in the blood plasma of patients with brain tumor (72). However, due to the aggressiveness and highly angiogenic nature of GBM, the search for CPs has turned to angiogenesis-related proteins. In a study conducted by Chiorean et al., angiogenesis and inflammation-related vascular endothelial growth factor (VEGF), platelet derived growth factor BB (PDGF-BB), insulin-like growth factor-1 (IGF-1), transforming growth factor beta (TGF-β), tumor necrosis factor-alpha (TNF-α), interleukin-6 (IL-6) and interleukin-8 (IL-8) levels were measured in preoperative serum samples of 14 GBM patients and 32 healthy patients. Serum PDGF-BB, IGF-1, and IL-8 levels were increased in GBM compared to the controls. Reduced IL-8 levels were linked to the development of coagulation necrosis, while increased levels with the development of endothelial hyperplasia and elevated VEGF levels were related with the development of ischemic necrosis (73). VEGF serum and tissue levels have been reported to be significantly higher in GBM compared to the controls and even increased in patients with brain metastases (74). It has also been shown that this increase in serum and tissue levels in GBM is due to increased VEGF gene expression (74). One another CP showing high expression in GBM compared to healthy brain tissues is chitinase-3-like protein 1 (YKL-40). A meta-analysis study to determine its prognostic value in GBM was found that elevated YKL-40 expression was associated with a worse OS in patients. It was concluded that it may be a good predictive tool as a prognostic biomarker for GBM patients (75).

GFAP is the protein most commonly detected in GBM and shows high expression levels. It is considered an immunohistochemical marker, especially in determining whether the tumors have glial character. These proteins, found in the cytoplasm of astrocytes, function in myelinization and astrocyte-neuron connection as glial intermediate filaments and are the most valuable indicator for neoplastic astrocytomas (76). Serum GFAP levels have been reported to correlate with tumor volume and histopathological tumor characteristics (77). In the study by Pérez-Larraya et al., preoperative plasma levels of insulin-like growth factor-binding protein 2 (IGFBP-2), YKL-40, and GFAP were measured in GBM patients. The diagnostic and prognostic values of IGFBP-2, GFAP, and YKL-40, both alone and as a combined biomarker profile, have been investigated, and it has been reported that a biomarker profile consisting of preoperative IGFBP-2, GFAP, and YKL-40 levels may be a helpful tool in the diagnosis of inoperable brain lesions with suspected GBM. In addition, it was concluded that IGFBP-2 levels can be considered an independent prognostic factor in GBM patients (78).

Determining the biomarker properties of proteins released into the circulation by GBM cells is of great importance for diagnosing the disease, monitoring response to treatment, and detecting relapses. However, the fact that the content of CPs varies depending on the character and localization of the tumor and their low concentrations makes detecting these proteins difficult. For these reasons, a standard biomarker for clinical use has not yet been defined. Therefore, further studies identifying more sensitive and specific CPs specific to GBM are critical in diagnostic processes and prognostic evaluations.

Limitations of the Review

This review comprehensively examines circulating biomarkers in GBM, but several limitations should be acknowledged. The review predominantly relies on existing literature, which may include studies with limited sample sizes and varying methodologies, leading to inconsistencies in findings. The heterogeneity of GBM and the complexity of biomarker analysis present challenges in drawing generalized conclusions. Moreover, the lack of large-scale clinical trials and standardized protocols for biomarker detection further restricts the ability to provide definitive recommendations for clinical practice. Additionally, while the review discusses various biofluids, a more focused comparison of their diagnostic utility would have strengthened the analysis.

Strengths of the Review

This review successfully highlights the transformative potential of circulating biomarkers in GBM diagnosis and monitoring. One of its major strengths lies in the comprehensive coverage of different biomarker types, including CTCs, cfNAs, cEVs, and CPs. Integrating preclinical and clinical evidence provides a well-rounded perspective on their current and future applications. Furthermore, the review emphasizes the clinical challenges and technical barriers, offering valuable insights into areas that require further research. The detailed exploration of emerging technologies and novel approaches adds depth and relevance, making this review a valuable resource for researchers and clinicians.

CONCLUSION and FUTURE PERSPECTIVES

The high incidence and mortality rates of brain tumors make the development of minimally invasive techniques

for the diagnosis and follow-up of both primary and metastatic tumors an urgent necessity. Despite significant developments in understanding the pathogenesis of GBM, patients still face low survival rates and limited treatment options. The current diagnostic process of GBM relies heavily on imaging modalities and TBs. However, this standard protocol has several limitations, including the inability to accurately represent the tumor, to assess tumor activity in real-time, and the surgical risks of repeated biopsies.

LBs offer many advantages over existing approaches. In particular, they provide reproducible sampling with a noninvasive method and allow tumor-associated molecules to enter the circulation in cases of increased permeability of the BBB. LBs have shown promise in the diagnosis and prognostic evaluation of GBM by providing valuable information before the clinical progression of the tumor. Blood, CSF, urine, and other body fluids carry tumor-associated biomarkers, including CTCs, ccfNA, EVs, and CPs. Studies in the literature show that these biomarkers are present in GBM patients, and their mutation profiles represent the origin of the tumor. This review provides a comprehensive summary of the available literature evaluating the role of biomarkers in the pre-diagnostic process and in monitoring response to treatment. It also sheds light on future research areas for discovering and validating GBM-specific biomarkers.

Integrating biomarkers with genetic and molecular profiling analyses is considered an important step toward more detailed monitoring of tumors. Current research suggests that the features of GBM that develop resistance to treatment can be better predicted by evaluating genetic mutations and biomarkers. In recent years, the correlation of ccfDNAs and ccfRNAs with tumor size and the tumor biology-reflective properties of EVs and CPs suggest that these biomarkers are promising tools for diagnosis and monitoring response to treatment. In particular, the presence of high levels of certain microRNAs associated with high-grade gliomas in treatment-resistant patients is valuable as a prognostic tool in the clinical management of GBM patients. However, several challenges remain that limit the clinical utilization of circulating biomarkers. These challenges include low concentrations of biomarkers, lack of standardized sampling and analysis methods, and the need to improve the specificity and sensitivity of biomarker detection. In the future, to overcome these challenges, larger-scale validation studies, standardization of detection techniques, and prospective studies to develop economically feasible methods should be conducted.

DECLARATIONS

Ethics committee approval: The authors of this review declare that there are no ethical concerns or conflicts of interest associated with this work. All contributors have adhered to ethical research and publication standards, and no part of this study involved activities that could present ethical issues.

Financial disclosure: The authors affirm that no financial or personal relationships exist that could be perceived as conflicts of interest in the preparation or publication of this review. All decisions and interpretations were made independently, without any influence from funding agencies, commercial entities, or not-for-profit sectors.

Author contributions: All authors confirm their active participation in the design, execution, and analysis of this review. They have collectively contributed to the development of the manuscript and have reviewed and approved the final version for publication.

Conflicts of interest: None.

Acknowledgments: None.

AI: Not applied

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