Detection of glioblastoma in biofluids

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The detection of glioblastoma (GBM) in biofluids offers potential advantages over existing paradigms for the diagnosis and therapeutic monitoring of glial tumors. Biofluid-based detection of GBM focuses on detecting tumor-specific biomarkers in the blood and CSF. Current clinical research concentrates on studying 3 distinct tumor-related elements: extracellular macromolecules, extracellular vesicles, and circulating tumor cells. Investigations into these 3 biological classifications span the range of locales for tumor-specific biomarker discovery, and combined, have the potential to significantly impact GBM diagnosis, monitoring for treatment response, and surveillance for recurrence. This review highlights the recent advancements in the development of biomarkers and their efficacy for the detection of GBM.

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BIOFLUID-based detection of glial tumors offers multiple approaches for improving quality of life in patients with glioblastoma (GBM). Many screening approaches take advantage of a slow initial phase of tumor growth for early detection, before a tumor has reached a greater degree of malignant potential. For example, in the more common malignancies such as breast and colon cancer, the early discovery of solid tumors with the development of mammography and colonoscopy screenings is a well-established clinical paradigm. In terms of malignant glial tumors (such as GBM), this analogy would extend to the concept of detecting a low-grade tumor prior to its progression to a high-grade glioma (secondary GBM). In patients with GBMs that form *de novo* (primary GBMs), biofluid screening would likely not be beneficial, and would instead be used in assessing prognosis, therapeutic monitoring, and surveillance of recurrent disease.

To extend survival and improve monitoring of patients with brain tumors, we need a detection strategy that provides 3 independent assessments: the ability to detect tumors early in the disease process, the ability to monitor current therapeutic modalities, and the ability to detect recurrence. Despite the great utility of imaging in evaluating GBM patients, the ongoing limitations of imaging to definitively diagnose and monitor both primary and secondary GBMs points to the need for more cost-efficient and effective biomarkers.³⁴ Thus, researchers and clinicians are

pursuing serum and CSF biomarkers to detect GBM early enough to provide timely treatment in secondary GBMs, which could potentially enable greater survival benefit, and enable one to follow disease progression and monitor chemotherapy and radiation treatments in primary GBMs.

Rationale for Biomarker Development

Analyzing markers of malignancy in biofluids was initially established in colorectal cancer with the detection of elevated serum carcinoembryonic antigen levels.¹⁶ However, given that levels of this normal physiological protein were not always elevated, and that it is associated with various other types of cancer, its use as a diagnostic biomarker is limited. Biomarkers for other cancers soon followed, such as prostate-specific antigen for prostate cancer and cancer antigen 125 for ovarian cancer, but analyses using these markers fail to meet diagnostic test requirements due to relatively low specificity.^{4,48} There are currently more than 20 FDA-approved biomarkers for tumors, the majority of which are only used to monitor response to therapy and progression of disease.¹⁵ Currently, GBM does not have a reliable biomarker in the serum or CSF, and initial detection relies on clinical diagnosis after the presentation of symptoms caused by the location of the tumor within the brain and its relative size (i.e., aphasia, seizures, paralysis, etc.). Thus, the ability to detect GBM early is vital

ABBREVIATIONS CTC = circulating tumor cell; ctDNA = circulating tumor DNA; EGFR = epidermal growth factor receptor; EM = extracellular macromolecule; EV = extracellular vesicle; GBM = glioblastoma; GFAP = glial fibrillary acidic protein; HGG = high-grade glioma; IDH1 = isocitrate dehydrogenase 1; IL = interleukin; LOH = loss of heterozygosity; MBP = myelin basic protein; MGMT = O⁶-methylguanine methyltransferase; miRNA = microRNA; PTEN = phosphatase and tensin homolog; T-reg = regulatory T cell; VEGF = vascular endothelial growth factor; 2-HG = 2-hydroxyglutarate.

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for clinicians to initiate treatment before patients become symptomatic, likely improving progression-free survival and quality of life.

In a future paradigm of GBM therapeutic management, early detection may be beneficial in 2 ways. First, the tumor volume would be smaller if discovered early, resulting in a smaller resection cavity and less impact on adjacent structures compared with the resection of a larger tumor. Second, smaller tumors may have less genetic variation between tumor stem cells and progeny, which can have a significant impact on sensitivity to chemotherapy and radiation.^{3,36} Cells within larger tumors are more genetically diverse, which can facilitate the evasion of immune responses, resistance to therapy, and initiation of recurrence. With regard to the clinical relevance of early detection of smaller tumors, there are studies both supporting and refuting the claim that tumor size correlates with prognosis and improved outcomes.^{6,7,19,40} However, more important than the early detection of small tumors may be the ability to diagnosis genetically favorable tumors such as isocitrate dehydrogenase 1 (IDH1) mutant tumors, which often present with seizures^{12,46} regardless of size, and have a long latent period as lower grade tumors. These tumors may be particularly sensitive to the combination of gross-total resection and chemoradiation.

The presence of certain mutations and cell surface markers in GBM may provide targets for detection in the serum and CSF. Given that the brain is bathed in CSF, biomarkers would theoretically be more prevalent in this biofluid compared with serum. Additionally, the blood-brain barrier may filter out potentially diagnostic macromolecules. The collection of CSF, however, is a more invasive process than blood collection; thus, identifying a biomarker in the serum would be preferable. Because every GBM is genetically distinct, some responding differently to chemotherapy and radiation regimens, the ability to distinguish between the molecular subtypes of GBM to direct tumor-specific therapy is also ideal for a tumor biomarker. GBMs harbor certain aberrations depending on their subtype, many of which can be detected by molecular profiling as well as by the analysis of extracellular vesicles (EVs) and circulating tumor cells (CTCs; Table 1). Thus, the discovery of a reliable “liquid biopsy” utilizing the serum and/or CSF will not only improve the ability to diagnose GBM, but also impact therapeutic strategies and surveillance of recurrence.

Current Evidence for Biomarkers

Extracellular Macromolecules (nucleic acids, proteins, and metabolites)

Free nucleic acid species, including tumor-specific genetic aberrations, can be detected in the blood and CSF of patients with GBM, which may be useful in ruling out disease in normal subjects. Additionally, these nucleic acid signatures may be able to differentiate between the various GBM subtypes, provide prognostic information, and guide tumor-specific therapeutic strategies.

The presence of circulating tumor DNA (ctDNA) in the blood of brain tumor patients was recently reported by Lavon et al. and Majchrzak-Celińska et al.^{24,27} As tu-

mor cells begin to die, they release intracellular content, including ctDNA, which can be isolated from the serum and can provide molecular genetic information about the malignancy. The Lavon group examined serum samples from 70 patients with either high-grade gliomas or oligodendrogliomas, and found that loss of heterozygosity (LOH), as well as methylation status of tumor suppressor genes, can be determined by analyzing ctDNA.²⁴ Specifically, analysis of serum ctDNA was able to detect methylation of O⁶-methylguanine methyltransferase (*MGMT*) and phosphatase and tensin homolog (*PTEN*), as well as LOH in chromosomes 1p, 19q, and 10q. LOH and/or methylation was detected in 62 (89%) of the samples. When compared with the tissue gold standard, the sensitivity for these serum biomarkers to predict genetic aberrations in the tumor was 51% (LOH) and 55% (methylation), with a specificity of 100%.²⁴ Similarly, the Majchrzak-Celińska group analyzed methylation status in *MGMT*, *RASSF1A*, *p15INK4B*, and *p14ARF* in serum ctDNA of 33 patients with newly diagnosed CNS tumors, including 17 gliomas, 6 meningiomas, and 10 metastatic malignancies.²⁷ Of the 17 samples from glioma patients, 12 (71%) demonstrated methylation in at least 1 of the 4 tumor suppressor genes: *MGMT* (18%), *RASSF1A* (47%), *p15INK4B* (12%), and *p14ARF* (41%). When compared with the tissue gold standard, the sensitivity and specificity of these serum biomarkers to detect gene methylation were 50% and 100%, respectively.²⁷ The low concentration of tumor-specific ctDNA in the serum is likely responsible for the relatively poor sensitivity of these nucleic acid biomarkers for detecting intracranial tumors, and suggests that analysis of CSF may improve false-negative rates given the difference in concentrations.

Recently, CSF analysis has demonstrated significantly increased concentrations of tumor-specific ctDNA when compared with serum, as reported by Pan et al. and De Mattos-Arruda et al.^{8,32} The Pan group isolated ctDNA from the CSF of 7 patients with solid brain tumors, 6 of whom had detectable tissue-concordant mutations in at least one of the following genes: *NF2*, *AKT1*, *BRAF*, *NRAS*, *KRAS*, and *EGFR*. Although the concentration of total ctDNA was less in CSF compared with serum, the concentration of mutation-specific ctDNA was higher in the CSF.³² Similarly, the De Mattos-Arruda group investigated ctDNA from the CSF of 12 patients with solid brain tumors, and found that the mutant allelic frequency was significantly higher in CSF compared with serum. Mutations in *EGFR*, *PTEN*, *ESR1*, *IDH1*, *ERBB2*, and *FGFR2* were readily detected in the CSF ctDNA with a sensitivity of 58%, compared with 0% for serum.⁸ Together, the results of these studies indicate that analysis of ctDNA in the CSF may be used for early detection of intracranial brain tumors that harbor specific oncogenic mutations.

Due to the inherent heterogeneity of malignancies, not all tumor cells will express the same genetic mutations. Thus, profiling RNA expression in biofluids may provide a more accurate representation of the malignant process. Dong et al. investigated the microRNA (miRNA) profile in the serum of 3 patients with GBM and compared them to 3 age- and sex-matched healthy controls.⁹ Of the 752 serum miRNAs analyzed, they found that the patients with GBM had 115 that were significantly upregulated and 24

TABLE 1. Biomarker summary: studies investigating various biomarkers in the blood and CSF of patients with GBM

Authors & Year	Biofluid	Biomarker	Sensitivity (%)	Specificity (%)
EMs (nucleic acids)				
Lavon et al., 2010	Serum	<i>MGMT</i> & <i>PTEN</i> methylation	55	100
		LOH in 1p, 19q, 10q	51	100
Majchrzak-Celińska et al., 2013	Serum	<i>MGMT</i> , <i>RASSF1A</i> , <i>p15INK4B</i> , & <i>p14ARF</i> methylation	50	100
Pan et al., 2015	CSF	<i>NF2</i> , <i>AKT1</i> , <i>BRAF</i> , <i>NRAS</i> , <i>KRAS</i> , & <i>EGFR</i> mutations	NR	NR
De Mattos-Arruda et al., 2015	CSF	<i>EGFR</i> , <i>PTEN</i> , <i>ESR1</i> , <i>IDH1</i> , <i>ERBB2</i> , & <i>FGFR2</i> mutations	58	NR
Dong et al., 2014	Serum	Elevated miR-340, miR-576-5p, & miR-626; decreased let-7g-5p, miR-7-5p, & miR-320	NR	NR
Tang et al., 2015		Elevated miR-185	NR	NR
Lai et al., 2015	Serum	Elevated miR-210	NR	NR
Yue et al., 2016	Serum	Decreased miR-205	NR	NR
Teplyuk et al., 2012	CSF	Elevated miR-10b, miR-21, & miR-200	91	99
Drusco et al., 2015	CSF	Elevated miR-223, miR-451, & miR-711	NR	NR
EMs (proteins)				
Jung et al., 2007	Serum	GFAP > 0.05 µg/ml	76	100
Tichy et al., 2016	Serum	GFAP > 0.01 µg/ml	85	70
Kiviniemi et al., 2015	Serum	GFAP > 0.014 ng/ml	86	85
Hormigo et al., 2006	Serum	Elevated YKL-40 & MMP-9	NR	NR
Elstner et al., 2011	Serum	Elevated BMP2, CXCL10, & HSP70	96	89
Quaranta et al., 2007	Serum	Elevated EGFR	NR	NR
Szymaś et al., 1986	CSF	GFAP > 0.04 µg/ml	NR	NR
Nakagawa et al., 1994	CSF	MBP > 4.0 ng/ml	NR	NR
Sampath et al., 2004	CSF	Elevated VEGF	NR	NR
Shen et al., 2014	CSF	Elevated IL-6	NR	NR
EMs (metabolites)				
Mörén et al., 2015	Serum	Elevated cysteine, lysine, & 2-oxoisocaproic acid metabolites	NR	NR
Locasale et al., 2012	CSF	Elevated 2-HG, histidine, & tryptophan metabolites	NR	NR
EVs				
Noerholm et al., 2012	Serum	Decreased RPL11, RPS12, TMSL3, & B2M	NR	NR
Manterola et al., 2014	Serum	Elevated RNU6-1, miR-320, & miR-574-3p	87	86
Skog et al., 2008	Serum	Detection of EGFRvIII	NR	NR
		Elevated angiogenin, FGF-α, IL-6, TIMP-1, TIMP-2, & VEGF	NR	NR
Shao et al., 2012		Detection of EGFR, EGFRvIII, PDPN, & IDH1	85	80
Akers et al., 2013	Serum	Elevated miR-21	87	93
Figueroa et al. (unpublished)	CSF	Detection of EGFRvIII	60	98
Circulating tumor & immune cells				
Sullivan et al., 2014	Serum	Tumor cells: CD14-positive, CD16-positive, and CD45-positive	NR	NR
MacArthur et al., 2014	Serum	Tumor cells: <i>hTERT</i> probe	NR	NR
Fecci et al., 2006	Serum	T regulatory cells: CD4-positive, CD25-positive, CD45RO-positive, FOXP3-positive	NR	NR
Heimberger et al., 2008	Serum	T regulatory cells: FOXP3-positive	NR	NR

NR = not reported.

that were significantly downregulated. Specifically, they identified 3 miRNAs that were the most overexpressed (miR-340, miR-576-5p, and miR-626), and 3 miRNAs that were the most underexpressed (let-7g-5p, miR-7-5p, and miR-320).⁹ Other investigators have also shown that miRNA can be used as a serum biomarker for GBM. Tang et al. showed that levels of miR-185 were significantly increased in the serum of 66 patients with glioma, which returned to

normal levels after surgery, chemotherapy, and radiation.⁴³ Similarly, Lai et al. demonstrated that miR-210 levels in the serum of 136 patients with GBM correlated with tumor grade and patient outcomes.²³ Conversely, Yue et al. found that significantly decreased levels of miR-205 in the serum of 64 patients with glioma correlated with Karnofsky Performance Scale scores, tumor grade, and overall survival.⁴⁹ Although results from these studies are promising,

there are no reported investigations into the sensitivity and specificity of individual, or panels of, serum miRNA to diagnose GBM.

CSF analysis of miRNA in patients with GBM has also yielded some intriguing results. Teplyuk et al. found that miR-10b and miR-21 levels in the CSF were significantly increased in 19 patients with GBM.⁴⁴ Additionally, they showed that levels of miR-200 were only elevated in CNS metastases, which could enable clinicians to differentiate those lesions from primary malignancies. This miRNA trio was able to distinguish GBM and metastases from healthy controls with an accuracy of 91%–99%.⁴⁴ Similarly, Drusco et al. demonstrated that elevated levels of miR-223, miR-451, and miR-711 in the CSF can be used to distinguish GBM from other CNS tumors.¹⁰ Together, these studies suggest that CSF profiling of miRNA may not only be useful in diagnosing GBM, but also in distinguishing it from other malignancies in the CNS.

Much like profiling free miRNA, the search for protein biomarkers in GBM focuses on aberrations in levels of normal physiological secreted proteins, given that no tumor-specific proteins have been identified. This approach relies on detecting proteins secreted into the serum or CSF from malignant cells in hopes of identifying levels at which clinicians can reliably diagnose GBM.

The most widely studied protein in the serum of patients with GBM is glial fibrillary acidic protein (GFAP). This protein is an intermediate filament highly expressed in glial cells, and several reports have shown that elevated serum GFAP levels can be used to diagnose glial tumors such as GBM.^{20,22,45} Jung et al. analyzed the serum of 50 patients with GBM and found that GFAP levels greater than 0.05 µg/ml were 76% sensitive and 100% specific for diagnosing malignant gliomas. Additionally, this group showed that GFAP was not detectable in the serum of patients with non-GBM.²⁰ Similarly, Tichy et al. analyzed the serum of 33 patients with GBM, and found that GFAP levels greater than 0.01 µg/ml resulted in a sensitivity of 85% and a specificity of 70% for diagnosing GBM and differentiating between non-GBM brain tumors.⁴⁵ Lastly, Kiviniemi et al. analyzed the serum of 27 patients with high-grade gliomas (HGGs) and found that GFAP levels greater than 0.014 ng/ml yielded in a sensitivity of 86% and a specificity of 85% for diagnosing HGGs, and correlated with a lack of the IDH1 mutation.²² Together, these studies indicate that detection of elevated GFAP levels in the serum may be a useful biomarker for GBM diagnosis.

Several other studies have focused on different proteins related to GBM. Hormigo et al. analyzed the serum of 66 patients with active GBM and 10 patients in GBM remission, and found that elevated levels of YKL-40 and MMP-9 in the serum correlated with active tumors and decreased overall survival.¹⁸ Additionally, Elstner et al. showed that the serum protein profile of 23 patients with HGGs had elevated BMP2, CXCL10, and HSP70, yielding a sensitivity of 96% and a specificity of 89% for HGG diagnosis.¹² Lastly, the analysis of epidermal growth factor receptor (EGFR) levels in the serum appeals to GBM investigators because most of these tumors have increased DNA amplifications and RNA expression of wild-type *EGFR*.⁵ Quaranta et al. analyzed the serum of 35 patients with GBM, and found elevated EGFR levels compared with normal

controls, which was inversely correlated with overall survival.³³ These results indicate that analysis of these protein levels in the serum may provide a panel by which clinicians can reliably diagnose GBM.

Similar to the nucleic acid biomarkers, protein biomarkers in the CSF may be more concentrated than in the serum. Szymaś et al. analyzed the CSF of 72 patients with intracranial tumors, and found that GFAP levels greater than 0.04 µg/ml enabled differentiation from other CNS malignancies and normal controls.⁴² Similarly, myelin basic protein (MBP), which is unique to the CNS, is an intriguing CSF biomarker in patients with GBM. Nakagawa et al. analyzed the CSF of 7 patients with malignant gliomas and showed that MBP levels greater than 4.0 ng/ml correlated with active malignancies, which decreased after surgery and chemotherapy.³⁰ Growth factors and cytokines have also been identified as potential biomarkers in the CSF of patients with GBM. Sampath et al. demonstrated that 90% of patients with malignant gliomas had elevated vascular endothelial growth factor (VEGF) levels in the CSF, compared with normal controls.³⁵ Shen et al. analyzed the levels of 19 tumor-related proteins in the CSF and demonstrated that patients with GBM had significant increases in interleukin (IL)–6 levels compared with low-grade gliomas and normal controls.³⁸ Together, the results of these studies suggest that CSF proteins may have utility as GBM biomarkers.

Investigators have recently started studying metabolomics in the serum and CSF of patients with GBM. Mören et al. showed that increased levels of the cysteine metabolite in serum was found in patients with GBM, and that increased levels of the lysine and 2-oxoisocaproic acid metabolites in the serum were found in patients with oligodendrogliomas.²⁹ Additionally, elevated levels of myoinositol and hexadecanoic acid metabolites in the serum were found to be of prognostic value in predicting long-term survival.²⁹ Similarly, Locasale et al. analyzed 124 cellular metabolites in the CSF of 10 patients with malignant gliomas, and demonstrated that metabolites from the amino acid, central carbon, lipid, and pyrimidine metabolism pathways were significantly different compared with normal controls.²⁵ Interestingly, levels of 2-hydroxyglutarate (2-HG) were increased in patients with GBM, indicating the presence of the isocitrate dehydrogenase 1 (IDH1) mutation.²⁵ Additionally, elevated levels of histidine and tryptophan metabolites in the CSF can be used to differentiate primary GBM from recurrence.²⁵ These studies indicate that while metabolomic biomarkers are relatively new in the field of cancer, they provide valuable real-time information about the cellular energy state within the malignant process.

Extracellular Vesicles

The use of extracellular vesicles (EVs), derived from the serum and CSF, as a biomarker of GBM is not as well studied compared with extracellular macromolecules (EMs). However, free nucleic acids and proteins are prone to more degradation than EVs and their contents, given the relatively harsh extracellular environment in the serum, and even CSF. EV secretion is a normal physiological process that has been described for nearly every type of cell,

and itself is a broad term that encompasses microvesicles and exosomes.²¹ Microvesicles (200–500 nm in diameter) are released via cell membrane blebbing and can contain cytoplasmic elements such as mRNA and miRNA. Exosome (40–100 nm in diameter) packaging and secretion, however, is more regulated and involves the intracellular endosomal system.²¹ Both microvesicles and exosomes can contain tumor-specific elements of the cell membrane, as well as nucleic acid species, which have utility as diagnostic biomarkers when isolated from the serum and CSF of patients with GBM.

EVs are known to contain an appreciable amount of nucleic acid species that can be profiled as biomarkers for GBM. Noerholm et al. analyzed serum-derived EVs from 10 patients with GBM and found significantly lower levels of 4 ribosomal function genes compared with normal controls: *RPL11*, *RPS12*, *TMSL3*, and *B2M*.³¹ However, no genes were identified in serum-derived EVs that were upregulated in patients with GBM.³¹ Similarly, Skog et al. showed that EGFRvIII mRNA is detectable in serum-derived microvesicles, which may direct receptor-specific adjuvant therapy.³⁹ Lastly, Manterola et al. found that increased levels of RNU6-1, miR-320, and miR-574-3p correlated with GBM diagnosis, yielding a sensitivity and specificity of 87% and 86%, respectively.²⁸ Together, these studies suggest that RNA profiling of serum-derived EVs can provide detailed transcriptomics of malignant cells to be used in GBM diagnosis.

Serum-derived EVs are also known to contain proteins that have biomarker potential in GBM. Skog et al. found that serum-derived EVs in patients with GBM had increased levels of angiogenin, FGF- α , IL-6, TIMP-1, TIMP-2, and VEGF.³⁹ This idea of developing a protein expression panel to diagnose GBM was then improved upon by Shao et al.³⁷ Using μ NMR technology, this group analyzed a panel of 4 GBM-related proteins (EGFR, EGFRvIII, PDPN, and IDH1) in serum-derived EVs, yielding a diagnostic sensitivity and specificity of 85% and 80%, respectively.³⁷ Additionally, this protein quad-panel had prognostic value in predicting how patients responded to temozolomide chemotherapy.³⁷ Thus, the relative abundance of tumor-related proteins within serum-derived EVs confers considerable diagnostic potential as biomarkers for GBM.

As with free-circulating EMs, CSF-derived EVs are more likely to be GBM-specific compared with those in the serum. Akers et al. analyzed EVs from the CSF of 13 patients with GBM and found an average 10-fold increase in miR-21 levels compared with nononcologic controls.² These results were then validated in a cohort of 29 patients with GBM, in which CSF-derived EVs yielded a diagnostic sensitivity and specificity of 87% and 93%, respectively.² Subsequently, this group also showed that while CSF-derived microvesicles contain more total RNA, CSF-derived exosomes were enriched with miRNA.¹ Of the 754 miRNAs analyzed in this study, 46 were present only in exosomes, while just 11 miRNAs were present in both microvesicles and exosomes.¹ The intriguing results of these studies suggest that miRNA profiling of CSF-derived EVs may have utility in GBM diagnosis.

Recently, investigators have also studied CSF-derived

EVs for the presence of GBM-specific genetic mutations. Figueroa et al.¹⁴ showed that EGFRvIII is detectable in CSF-derived EVs in a subgroup of 81 patients with GBM. Of the 25 patients (30.8%) who were EGFRvIII tissue-positive, 15 had detectable levels of EGFRvIII in CSF-derived EVs, yielding a sensitivity of 60%. Of the 56 patients (69.2%) who were EGFRvIII tissue-negative, only 1 patient had detectable EGFRvIII in CSF-derived EVs, yielding a specificity of 98%. Additionally, EGFRvIII-positive CSF-derived EVs had increased mRNA expression of wild-type *EGFR*, which is a known aberration present in the classical subtype of GBM.⁴⁷ Given these results, this novel diagnostic modality has potential for diagnosing a subpopulation of GBM via EGFRvIII status, as well as differentiating a GBM subtype.

Circulating Tumor and Immune Cells

The least researched approach to diagnosing GBM via biofluids is detecting CTCs. This strategy involves isolating primarily rogue tumor cells that separate from the primary malignant mass and enter the blood or CSF, but can also include subverted tumor stromal cells, such as immune and endothelial cells. Currently, CTCs have only been studied in the serum of patients with GBM, and no investigations identifying CTCs in the CSF have been reported.

Searching for CTCs in the serum of patients with GBM is difficult given that reliable tumor-specific cell surface markers have yet to be established. Thus, investigators have targeted panels of membrane proteins that may identify GBM-specific CTCs. Using a trio of antibodies (anti-CD14, anti-CD16, and anti-CD45) Sullivan et al. detected CTCs in the serum of 13 of 33 patients with GBM.⁴¹ CTC status was subsequently confirmed by FISH analysis using genetic aberrations known to be present in specific GBM subtypes: proneural (*ASCL1*, *DLL3*, *OLIG2*, and *SOX2*), neural (*SLC12A5* and *SYT1*), classic (*AKT2*, *EGFR*, and *GFAP*), and mesenchymal (*RELB*, *SERPINE1*, and *TGFBI*).⁴¹ Similarly, MacArthur et al. isolated CTCs from the serum of 8 of 11 patients with GBM using an adenoviral probe to *hTERT*, which is elevated in this malignancy.²⁶ CTC status was subsequently confirmed by FISH analysis for the overexpression of wild-type *EGFR*, GFAP, and nestin, and the absence of EpCAM.²⁶ The promising results of these studies indicate that detecting CTCs in the serum is possible in patients with GBM, and may prove to be a viable diagnostic strategy in the future.

The detection of tumor-associated immune cells in the blood has also been described in patients with GBM. Immune system evasion and manipulation are hallmarks in cancer, and are well described in malignant glioma. Several groups have focused their efforts on identifying the increasing regulatory T-cell (T-reg) population in the peripheral blood of patients with GBM. T-regs are known as immune system suppressors and may act to decrease the immune response to a growing malignancy. Fecci et al. showed that while absolute counts of CD4-positive T cells and T-regs decreases over time in patients with GBM, the fraction of T-regs in the remaining CD4-positive cells increases in the blood.¹³ Similarly, Heimberger et al. found that T-regs were not present in regular brain or low-grade gliomas, but only in malignant gliomas, suggesting that

their presence is detectable in the serum.¹⁷ Together, these studies indicate that evaluating a patient's serological immune response to GBM could be a method of monitoring and/or diagnosing HGGs.

Summary

Analyzing biofluids to diagnose GBM earlier in the malignant process is an ever-evolving area of interest in neurooncology. Investigators have discovered numerous free nucleic acid and protein signatures in the serum and CSF that are paving the way for diagnostic biomarkers. The field of EV isolation and content characterization is expanding and improving, providing another potentially diagnostic approach. Finally, additional research is required to more effectively detect CTCs, which have proven to be an intriguing and promising modality for GBM diagnosis. These novel approaches may not only provide tumor-specific information that can direct individualized therapeutic strategies, but may also be used to screen for secondary GBMs, monitor therapeutic efficacy in primary GBMs, and survey for recurrent disease. However, to reach the stage of a practical and effective assay for detection of GBM, greater sensitivity and specificity of newer "liquid biopsy" techniques is required. Additionally, because obtaining CSF involves a semi-invasive procedure, the "holy grail" would be to develop a blood-based assay that reduces patient risk. If a low-cost and robust serum biomarker can be identified, then early diagnosis of secondary GBM is viable, and active monitoring of both primary and secondary GBM would be facilitated. For now, current assays are limited in scope, although with the demand and funding for such diagnostic and screening tools increasing, patients may be able to benefit from these advances in the near future.

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Disclosures

The authors report no conflict of interest concerning the materials or methods used in this study or the findings specified in this paper.

Author Contributions

Drafting the article: both authors. Critically revising the article: both authors. Reviewed submitted version of manuscript: both authors. Approved the final version of the manuscript on behalf of both authors: Carter.

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