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# Diagnosis of CNS Lesions by CSF

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ABSTRACT

CNS tumors include primary and metastatic neoplasms, which together account for about 1% of human body tumors. The fragile nature of brain and spinal cord parenchyma limits both diagnostic and therapeutic approaches. Fortunately, the recently developed liquid based biopsy (LBB) technique provide highly convenient, fast and less-invasive method to collect and test the potential biomarkers. Biomarkers derived from liquid biopsies can promptly reflect changes on the gene expression profile of tumors. Biomarker derived from tumor cells contain abundant genetic information, which may provide a strong basis for the diagnosis and the individualized treatment of brain tumor patients. CSF can be used as a resource of biomarkers, the sensitivity and specificity of CSF biomarkers of patients with brain tumor is typically higher than those detected in peripheral blood and other sources. This chapter reviews the current different biomarkers in CSF and their significance in brain tumor diagnosis and monitoring.

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## Introduction

An accurate, non-invasive diagnostic test for brain tumors is currently unavailable, and the methods of monitoring disease progression are not fully reliable [1]. CSF is produced by the choroid plexus in the ventricular system of the brain and is absorbed by the arachnoid granulation of the dura sinus and flows back to systemic circulation. CSF circulates through CNS, in fact. The whole CNS (brain and spinal cord) is soaked in CSF. Physically, CSF function as a buffer for protection of physical damage to CNS. Due to close contact relation between CNS and CSF, any changes in CNS, no matter pathological or physiological, will reflex in CSF.

## Liquid-based biopsy (LBB)

Almost 150 years ago in 1869, the pathologist Thomas Ashworth provided first evidence for the presence of circulating tumor cells (CTC) in the blood of a patient with metastatic cancer and, described for the first time a phenomenon nowadays considered as liquid biopsy, some people referred as liquid based biopsy (LBB) [2]. The concept of liquid based biopsy aims at simple, fast, and cost efficient monitoring of disease status or response to treatment. Here, LBB offers several advantages compared to "conventional" tissue biopsy: LBB is less burdensome than a tissue biopsy, because body fluids like blood, saliva or urine are much easier to access. For some diseases such as lung cancer, taking a tissue biopsy is clinically often not possible, e.g. due to a high risk of bleeding, nerve injury or disease spreading [2]. Moreover, tissue biopsies may not appropriately reflect the complex molecular profile of a primary tumor, because of its intratumoral or spatial heterogeneity, which can only be addressed by taking multiple biopsies from different tumor areas [2]. Compared to this, LBB may offer a more comprehensive cross-section of heterogeneous diseases. Furthermore, LBB may also provide insights into molecular drivers of different primary or metastatic tumors, which may significantly differ in the same patient. Since the genome of tumor cells is often highly unstable and susceptible to changes under different selective pressure (like chemotherapy), LBB may allow longitudinal disease surveillance to monitor developing tumor heterogeneity. Overall, the LBB concept complements the personalized medicine approach and provides an innovative way for patient selection in clinical trials. Here mutational analysis supports patient eligibility for target treatment [2]. For example, the traditional Pap smear has been replaced by LBB, which provides much better cellular preservation and much clearer cell morphology. It becomes a routine clinical procedure for collecting cervical tissue for cytologic examination. LBB is not only the cytology examination of body fluid, but also the biomarker

detection from body fluid. Other blood components recently introduced, as blood-based biomarker sources, like exosomes or platelets, will focus on ctDNA and CTCs. All these analyses can be used to increase our knowledge about the underlying disease (e.g. tumor burden and heterogeneity) and ultimately translating into improved cancer diagnosis, therapy guidance and disease surveillance [2].

Another example of LBB is urine cytology plus cytogenetic study (UroFish), Fluorescence in situ hybridization (FISH) is a powerful tool to detect extra copies of chromosomes 3, 7 and 17 or deletion in four or more cells collected from the urine of the patient, since extra copies of chromosomes 3, 7 and 17 are usually seen in urothelial carcinoma.

Also homozygous deletion of 9p21 should be observed in 12 or more of the cells examined. Those above cytogenetic changes are commonly seen in urothelial carcinoma. This test has not only skip the invasive and painful cystoscopy procedure, but also much cheaper and safe. In addition, Chromosome 1p/19q co-deletion is currently a diagnostic feature for oligodendroglioma (See Fig. 1)

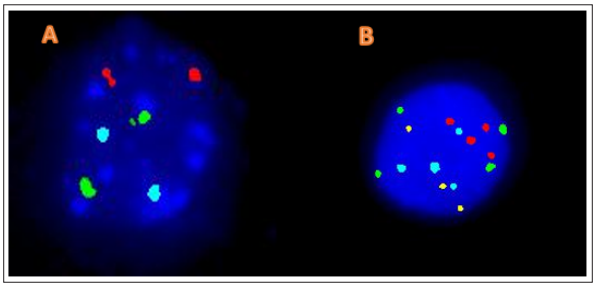


**Figure 1:** Positive for deletion of 1P (A), Positive for deletion of 19q (B).

CSF, as one of body fluid, is an important source of potential molecular biomarkers, mostly collected by lumbar puncture (LP) or surgery around the brain area. For example, CSF contains various biomarkers, such as ctDNA, miRNA, proteins, and EVs, which are typically derived from brain tumor cells. Tumor cells usually co-exist with their microenvironment. Therefore, tumor-related biomarkers can be more prominent in CSF nearby the site of the disease.

**CSF is collected by a procedure called lumbar puncture (LP), which is a minimal invasive procedure.**

Indications for lumbar puncture

1. To obtain pressure measurements and collect a sample of the CSF for cellular, cytological, chemical, and bacteriologic examination.
2. To aid in therapy by the administration of spinal anesthetics and occasionally, antibiotics or antitumor agents, or by reduction of CSF pressure.
3. To inject a radiopaque substance, as in myelography, or a radioactive agent, as in radionuclide cisternography.

Lumber puncture (LP) carries some risks if the CSF pressure is very high, which may increases the possibility of a fatal cerebellar or transtentorial herniation. The risk is considerable when papilledema is the result of an intracranial mass, but it is much lower in patients with subarachnoid hemorrhage, in hydrocephalus with communication between all the ventricles, or with pseudo tumor cerebri, conditions in which repeated LPs may at times be employed as a therapeutic measure. Asymmetric lesions, particularly those near the tentorium or foramen magnum carry a great risk of herniation precipitated by LP. In patients with purulent meningitis, there is also a small risk of herniation, but this is far outweighed by the need for a definitive diagnosis and the institution of appropriate treatment at the earliest moment. With this last exception, LP should generally be preceded by CT or MRI whenever an elevation of intracranial pressure is suspected.

CSF contains various biomarkers, such as ctDNA, miRNA, proteins, and EVs, which are typically derived from brain and spinal cord tumors [1]. CSF is usually considered as an extension of the extracellular compartment within CNS due to the close relation of each other. Due to the presence of blood brain barrier, brain tumors' biomarkers are most low or even undetectable in blood sample. In that case, CSF is a suitable repository of clinical biomarkers, and increasing studies have reported that CSF-derived biomarkers are more abundant than those in the peripheral blood and other sources [2]. For example, ctDNAs derived from brain tumor cells are more abundantly present in the CSF than in the plasma [1]. In addition, CSF is a better source of circulating nucleic acids than the plasma from brain tumor patients [1]. One study composed by 8 brain tumor patients, indicated that the detection of tumor-specific mutations in CSF ctDNA has higher sensitivity when comparing with plasma ctDNA (100% vs 37.5%, respectively) [3,4]. Although plasma is still a more common and convenient source for the quantitative isolation and detection of nucleic acid, CSF becomes a more qualitative source for collection of nucleic acids [4,5]. CSF may provide a less invasive diagnosis and treatment monitoring of brain tumor patients [6]. Currently, liquid biopsy techniques including Enzyme-linked immunosorbent assay (ELISA), Polymerase Chain Reaction (PCR), and Next-Generation Sequencing (NGS) have been standardized for the detection of potential CSF biomarkers. Based on these techniques, changes on the expression of ctDNA, miRNA, proteins, and EVs from brain tumor cells can be examined in the CSF and, more precisely, translated into the diagnosis and treatment, as well as monitoring recurrence and treatment response of brain tumors [1].

One of important clinical issue is to determine the new growth on the surgical bed (6 months or more after brain surgery for resection of a brain tumor followed by standard chemo-radiation therapy) is a recurrent tumor verse reactive changes, like radiation necrosis. Unfortunately, the current MRI scans cannot distinguish these two lesions. While CSF may play an important role in this critical issue as lumbar puncture collected, CSF may contain mutated tumor DNAs. Increased mutated tumor DNA may suggest recurrent tumor. In addition, protein analysis may solve this difficulty by measuring the proteins in CSF, tumor-related protein (like glial fibrillary acid protein, GFAP) verse reactive related (like macrophages marker of CD68) protein to determine the new growth is tumor or non-tumor. In this case, not only an invasive surgical procedure may be avoided but also expensive multiple brain MRI scans may be avoided.

**Brain tumors related CSF biomarkers**

Tumor in CNS roughly divided into two group, primary and secondary. Primary brain tumor is those tumor occurs in brain and spinal cord parenchyma, primarily glial neoplasm, like adult astrocytomas graded by WHO grades from II to IV; and oligodendrogliomas from grade II to III; as well as Ependymomas from grade II to III. Secondary tumors indicates those tumor original from outside CNS and metastatic to the brain, also called metastatic brain tumors. Like lung cancer, breast cancer and melanoma. Metastasis is one of the characteristics of malignant neoplasms.

**CtDNA**

CtDNA is referred to the DNA that comes from tumor cells and stably circulates in body fluids. Tumor-derived ctDNA have been extracted from CSF samples of patients with brain tumors, and a series of genes mutation have been assessed [7,8]. Interestingly, one particular study has been demonstrated that CSF-derived ctDNA can better reflect sequence mutations in driving genes when compared to plasma ctDNA [9]. Two new ways have been utilized to detect genetic mutations: droplet-digital PCR (ddPCR) and NGS [10-12]. One study has performed ddPCR with targeted amplican sequencing to search for mutations in CSF ctDNA of primary and metastatic brain tumor patients [13]. A number of tumor gene mutation were detected in CSF-derived ctDNA from 7 patients with solid brain tumors, where 6 had detectable tumor mutations in at least one of the following genes: NF2, AKT1, BRAF-V600, NRAS, KRAS, TP53, and EGFR [14]. Interestingly, gene mutations in RGS12, CASR, AQR, MTMR4, and KDM6A were detected in CSF-derived ctDNA from medulloblastoma patients [8]. In addition, CSF-derived ctDNA were extracted from 53 patients to study alterations in 341 cancer-associated genes by NGS, and somatic alterations were detected in more than half of patients with primary and metastatic brain tumors, but not detected in patients without brain tumors [11].

Gene mutations in *IDH1, TP53, EGFR, PTEN, FGFR2* and *ERBB2* have been detected in CSF-derived ctDNA of patients with glioblastoma (GBM). Other glioma associated gene mutations such as *IDH1/2, TP53, ATRT, TERT, H3F3A,* and *HIST1H3B*, have been detected in CSF ctDNAs and equally contributed in diagnosis and treatment of diffuse gliomas patients [6]. Similarly, even this newly recognized brainstem gliomas and the so-called midline gliomas, their special mutation, *H3F3A* and *HIST1H3B* have been detected in CSF-derived ctDNA of those patients [15, 16].

Outcomes for individuals with CNS malignancy remain abysmal. A major challenge in managing these patients is the lack of reliable biomarkers to monitor tumor dynamics. Consequently, many patients undergo invasive surgical procedures to determine disease status or experience treatment delays when radiographic testing fails to show disease progression. Wang, et al showed that primary CNS malignancy shed detectable levels of tumor DNA into the surrounding CSF, which could serve as a sensitive and exquisitely specific marker for quantifying tumor burden without invasive biopsies. Therefore, assessment of such tumor-derived DNA in the CSF has the potential to improve the management of patients with primary CNS tumors [8]. Similarly, *MYD88* mutation, an unique mutation for lymphoma, has been detected in CSF extracted from patients with primary central nervous system lymphoma (PCNSL) [16, 17]. Gene mutation in *MYD88* has also been detected in CSF-derived ctDNA of one patient with secondary central nervous system lymphoma (SCNSL) [18-20]. Another genes whose mutations have diagnostic potential, such as *CD79B*, were found in PCNSL patients [21] Interestingly, several studies have also shown that neither *CD79B* nor *MYD88* mutations have been found in glioma patients [22, 23]. Therefore, *CD79B* and *MYD88* mutations, which were detected in CSF-derived ctDNAs, may play an important role distinguishing lymphoma from other brain tumors [24]. In that case, *MYD88* and *CD79B* could be potentially used as molecular signatures for lymphoma [1].

MYD88 is an adaptor protein encoded by the myeloid differentiation primary response protein 88 (*MYD88*) gene. The *MYD88* gene was discovered in the 1990s as a primary differentiation response gene in myeloid precursors. MYD88 protein contains three main structures: a death domain (DD) at the N terminus, an intermediate linker domain (ID), and a Toll/interleukin-1 receptor domain (TIR) at the C terminus [19]. *MYD88* protein transfers outside signals to certain proteins called Toll-like receptors and interleukin-1 (IL-1) receptors, and then activates the nuclear transcription factor κB (NF-κB) signaling pathway [20]. NF-κB regulates multiple genes, such as genes controlling immune responses and inflammatory reactions and pro-survival genes. *MYD88* plays a central role in the innate and adaptive immune response [21]. Defects in this gene in patients can lead to increased susceptibility to pyogenic bacterial infections [22].

A missense mutation (L265P, position 265 changes from leucine (CTC) to proline (CCG)) in MYD88 is found in about 90% of Waldenström macroglobulinemia (WM), activated B cell type diffuse large B-cell lymphoma, and IgM monoclonal gammopathy of undetermined significance (MGUS) cases [23]. *MYD88* L265P mutation causes NF-κB activation [24]. It has been reported that there was a prevalence of *CD79B* and *MYD88* mutations, higher than that of systemic diffuse large B cell lymphoma [25].

This *MYD88* L265P mutation can be served as a diagnostic hallmark, as well as a potential therapeutic target. In lymphoma patients with this mutation, strategies have been devised to halt MYD88 oncogenic activation by targeting IRAK1/4, JAK, and BTK, or by disrupting the myddosome assembly [23]. The BTK inhibitor ibrutinib (also known as PCI-32765) reduces the binding of BTK to MYD88 L265P, and subsequently induces apoptosis of lymphoma cells) There have been clinical trials in patients with relapsed or refractory diffuse large B cell lymphoma, and refractory Waldenström macroglobulinemia [26]. *MYD88* L265P mutation can be detected by using DNA sequencing including next-generation sequencing (NGS) and polymerase chain reaction (PCR) study.

Wang, et al demonstrated that cell-free DNA shed by cancer cells has been shown to be a rich source of putative tumor-specific biomarkers. Because cell-free DNA from brain and spinal cord tumors cannot usually be detected in the blood, probably due to the brain blood barrier. It has been found that CSF that baths the CNS is enriched for tumor DNA, and referred as CSF-tDNA. 35 cases of primary CNS malignancies have been studied and at least one mutation in each tumor has been identified by using targeted or genome-wide sequencing [8]. In this study, it was found that high-grade (WHO grade III and IV) tumors were more likely to have detectable CSF-tDNA than low-grade lesions, which was evidenced by the fact that all but one high grade tumor (18, 19) was detected. The levels of CSF-tDNA were also higher in high-grade lesions than in low-grade lesions [8].

**MiRNAs**

The role of microRNA (miRNA) in tumorigenesis is a relatively new and exciting field of research. MiRNAs are small, non-coding RNAs (around 22 nucleotides in length) that can bind mRNA to affect translation of genes, which can regulate oncogenes and tumor suppressor genes and thus are associated with tumor growth, including cellular proliferation, angiogenesis, invasion and apoptosis [27]. MiRNA can be released from brain tumor cells [28]. Free miRNAs possibly result from death of tumor cells or secretion of tumor cells, leading to the release of nucleic acids in the extracellular matrix. The main function of miRNAs includes the modulation of gene expression by mRNA silencing and/or degradation. Interestingly, a single mRNA may be able to target several mRNAs simultaneously (pleiotropic effect) [28, 29]. The association between miRNAs and brain tumorigenesis was first introduced in 2005. Three years later, the presence of miRNA in

circulating body fluids from patients with brain tumor was finally detected [30]. However, due to the blood-brain barrier, it has been hypothesized that miRNA present in the CSF can better reflect the brain physiology and pathology more accurately than plasma miRNA [31,32]. Several studies have demonstrated the causes and significance of extracting miRNAs from CSF [14, 32-34]. Therefore, due to the presence of RNA-degrading enzymes in the blood, the expression/secretion of miRNA in the CSF appears to be more define and more accurately to reflect the malignant process of brain tumors [35,36].

Glioma associated miRNAs include miRNA-21, miRNA 181a and miRNA 181b, miRNA 128 as well as miRNA 221/222 [27]. Other related findings include that meningioma and brain metastasis showed elevated expression of miR-935, while miR 935 expression is absent in lymphoma and gliomas [1]. In addition, miR-200 levels were solely elevated in brain metastases, but no under other pathological conditions, which allow the discrimination between GBM and metastatic brain tumors. Actually, current MRI scanning hard to separate GBM from metastatic brain tumors, especially when only one metastatic nodule present. Comparative analysis of these particular miRNA allowed the distinction of GBM and metastatic brain tumor from healthy controls, with an accuracy of 91-99% [32]. Similarly, miR-451 and miR-711 are upregulated in meningiomas, gliomas, and medulloblastoma while downregulated in lymphomas. In particular, miR-125b and miR-223 are important diagnostic biomarkers for GBM, medulloblastoma, and brain metastasis. Therefore, differential miRNA expression can be used as a unique approach for the minimally invasive diagnosis of GBM [1-33]. A study including 118 patients with different types of brain tumors and non-neoplastic neuropathologies demonstrated that by quantitative reverse transcription PCR analysis, the level of miR-10b and miR-21 are found significantly increased in CSF of patients with glioblastomas and brain metastasis of breast and lung cancer, compared with tumors in remission and a variety of non-neoplastic conditions. Another study revealed that miR-15b and miR-21 were differentially expressed in CSF samples from patients with gliomas, compared to control subjects with various neurological disorders [33]. MiRNA can be detected by methods like Northern blot and microarrays, as well as quantitative RT-PCR [27]. In addition, melanoma has a strong tendency to metastasize to the brain. It is estimate that almost 99% end stage melanoma patients have brain metastasis. CSF cytology is often used to search for melanoma-derived brain metastases. However, this procedure is not sensitive enough to diagnose this metastatic subtype [30]. Fortunately, it has been observed that the presence of three mRNA markers in the CSF, MAGE-3, MART-1 and tyrosinase – may be able to diagnosis melanoma brain metastasis [1]. Nevertheless, the clinical utility of miRNA as CSF biomarkers has not been validated yet. Further research aiming the detection of miRNA in the CSF of patients with melanoma-derived brain metastases is still warranted [1].

**Extracellular Vesicles (EVs)**

Extracellular vesicles (EVs) are nanometer size membrane-closed particles that contain a variety of miRNA [37-41]. The incorporation of miRNA into EVs results in protection of miRNA from degradation in the biofluid environment [42]. Most of them range in size from 30nm to 1000 nm. Based on the size, biogenesis, and biophysical characteristics, EVs can be classified as exosomes, microvesicles and apoptotic bodies [42]. These vesicles are similar to multiple biologic processes and, at the same time, capable of promoting tumor progression [42-44]. EVs, sometimes referred to as "exosome", carry an abundant array of lipids, DNAs, miRNSs and proteins, which can be reflect their identity for analysis in liquid biopsy for brain tumor [45-48]. The secretion of extracellular vesicles from brain tumor cells is quite complicated. A number of studies have shown that EVs can be found and isolated from the CSF [43, 44] and, apparently, this procedure can be more feasible that isolating and sequencing exosomal miRNA from CSF [32, 33, 37]. Multiple CSF-related miRNAs have been found to be significantly associated with primary and metastatic brain tumors. Intriguingly, certain miRNAs may be upregulated in some brain tumors while downregulated in others, indicating that combination of miRNA signatures can be useful to distinguish different brain tumors [1]. CSF-derived EVs provide a platform for detection of tumor specific biomarkers in the brain. For instance, the analysis of mutant IDH1 in CSF-derived EVs of patients with glioma may play a new role for the diagnosis [49]. Moreover, the levels of miR-21 in CSF-derived EVs of GBM patients were, in average, 10-fold higher than the levels in control subjects, and miR-21 in CSF-derived EVs yielded a diagnostic sensitivity and specificity of 87% and 93% for GBM, respectively [44]. Another study also indicated that the miR-21 signature from CSF-derived EVs have a diagnostic significance for GBM patients [47].

Despite recent advances, in-depth validation of CSF-derived EVs as biomarkers is still expected. For this, acquiring CSF-derived EV samples from brain tumor patients in a large scale, is necessary, but will certainly require a coordinated multi-institutional effort [1].

**Proteins**

At protein level, CSF still is a valuable resource for biomarkers. For example. CSF electrophoresis to identify the oligoclonal band has been the gold standard for many years in diagnosis of demyelinative disease such as multiple sclerosis.

In the field of neurooncology, CSF represents an appropriate medium to obtain LBB, that can be informative for diagnosis, tumor classification and risk stratification. Proteomic profiling of pediatric CNS malignancies has identified putative protein makers of disease, yet few effective biomarkers have been clinically validated or implemented. Advances in protein quantification techniques have made it possible to conduct such investigations rapidly and accurately through proteome-wide analysis [2]. For instance, the level of glial fibrillary acidic protein (GFAP) were quantitatively determined in the CSF of brain tumor patients. Hence, it was observed that GFAP levels from GBM patients surpassed those from other brain tumor and cerebral lesions of distinct etiology [1].

Protein and peptides are particularly promising as biomarkers since they are the functional entities of biological processes and therefore, their expression levels typically correlate with disease pathology [50-69]. In addition, in comparison to other molecular attributes such as gene expression profiles or next generation sequencing strategies, protein biomarkers are more cost-effective for implementation in a clinical setting. Evolving technologies have enhanced the precision and expedience of proteome analysis. By exploring the protein content of body fluids in patients and controls, mass spectrometry (MS) has emerged as a promising approach for putative protein biomarker discovery. Application of MS have proven sensitive in quantifying proteomic profiles of CSF and identifying candidate biomarkers in neurological disease, particularly for neurodegenerative disorders [51-60]. Early studies suggested that this sensitivity could indeed facilitate biomarker discovery [3].

The utility of protein biomarkers in the arena of pediatric neuro-oncolgy is an emerging area of research and limitations should be carefully considered in order to advance promising and validated biomarkers to the clinic practice.

For example, ∝ fetal protein (AFP) and *β*hCG are biomarkers in patients with intracranial malignant germ cell tumors and can be used to measure response to therapy [57]. Both *A*FP and *B*hCG have been examined for their utility in CNS germinoma but high sensitivity was not established. For these tumors, histologic confirmation remains the gold standard [61]. Other reports demonstrate *B*hCG in the CSF to be highly sensitive and specific for diagnosing intracranial ectopic germinoma that arise in rare sites other than the pineal and suprasellar regions. Additional markers such as placenta alkaline phosphatase (PLAP) in the CSF have been shown to be clinically useful tumor markers in the diagnosis and monitoring of intracranial germ cell tumors [62].

A number of other putative CSF protein biomarkers for pediatric CNS malignancies have been reported. Identification of these proteins has, in many instance, led to greater insight into the pathogenesis of pediatric tumors, revealing novel roles of CNS proteins including hemaphorins, insulin-like growth factor binding proteins, metabolic proteins, and apolipoproteins, among others. The rigor of these studies is highly variable and the candidates identified must be subject to further validation before consideration for clinical utility [3].

Growth factors and cytokines have also been identified as potential biomarkers present in the CSF of GBM patients. About 90% of patients with malignant gliomas present elevated vascular endothelial growth factor (VEGF) levels in the CSF, which may correlate the vascular proliferation in histopathology of GBM. Additionally, related to CSF levels of elevated OPN, VEGF and C-C motif chemokine ligand (CCL) 4 levels in the CSF were significantly increased in glioma patients when compared with non-tumor controls [49]. One particular study has shown that fibroblast growth factor (FGF) and VEGF levels in patients with high-grade glioma were apparently higher than patients with low-grade glioma [50]. A further study has indicated that nerve growth factor (NGF) levels in the CSF elevate proportionally to the glioma grade [51]. The levels of 19 tumor-related CSF proteins have been examined, and results demonstrated that GBM patients have significant inceases in interleukin (IL)-6 levels compared with patients with low grade gliomas and normal subjects. Similarly, CSF IL-8 levels were also markedly inceased in astrocytic tumor patients compared to healthy controls [63-70]. Altogether, these studies suggest that CSF proteins have potential use as glioma biomarkers [1].

Several CSF-related protein biomarkers, such as CXC13, IL-10, IL-6, B2M, sIL-2R, sCD27, ATIII, OPN, Neoprotein, sTACI, sBCMA, APRIL, and BAFF, have a putative diagnostic value in lymphomas. The elevated CXC chemokine ligand (CXCL) 13 plus IL-10 was 99.3% specific for PCNSL and SCNSL, with a sensitivity significantly greater than standard CSF tests [1]. IL-10, IL-6, beta-2 microglobin (B2M), and soluble IL-2 receptor (sIL-2R) levels in CSF from patients with CNS lymphoma were apparently higher than non-lymphoma patients [52]. A specificity of 90% was also found for increased sIL-2R in the CSF, which correlated with the proper diagnosis of patients initially suspected of having PCNSL [53]. CD27 is a receptor molecule that integrates the tumor necrosis factor receptor (TNFR) superfamily and, as such, it may regulate the activation of B cell and synthesis of immunoglobulin [54]. A total of 42 CSF samples were collected from various types of brain tumor patients, and the results indicated that the levels of soluble CD27 (sCD27) were significantly higher in the PCNSL group when compared to controls with unrelated brain tumor [55].

Importantly, biomarker discovery studies have demonstrated a marked mismatch between the vast dynamic range of proteins in the blood against which Low-abundance protein markers must be detected, and the limitations of detection of analysis methods [56]. However, unlike serum, CSF is a metabolically active medium that contains a much less complex complement of proteins at concentrations 100 – 400 fold lower than those found in serum [64]. This property could render tumor-specific markers more distinctly measurable and easier to detect. Additionally, due to the high turnover rate of CSF, serial sampling at various stages during a patient's clinical course can enable efficient monitoring of distinct disease states, such as pre- and post- chemotherapy administration, or pre- and post-tumor resection, or for minimal residual disease (MRD) testing, such as in the case of leukemia [3].

However, there are limitations to the interpretation of CSF proteomic studies as well as the usage of CSF as a biomarker. For example, protein composition of CSF is dependent on patient attributes, such as age, the specific site of CSF access and the extent of blood contamination [66-68]. Differences in CSF proteome composition due to gender are predicted to be less relevant [65]. For pediatric patient, CSF specimens can be particularly constrained for analysis owing to the low protein concentration of CSF. Consequently, the number of samples needed to achieve statistical power to discover useful biomarkers and the amount of specimen available for subsequent validation experiment can be limited. Furthermore, tumor size and stage should be reported as they may affect disease-associated protein levels in the CSF. Finally, CSF biomarkers may not be informative for brain tumors that are not in proximaty to CSF, such as hemispheric astrocytoma, lobar gangliogliomas or dysembryoplastic neuroepithelial tumors (DNETs) [3].

**Conclusion**

CSF is a suitable medium for clinical biomarkers. Tumor cells exist in equilibrium with their microenviroment and accordingly, fold-differences in tumor-related markers relative to blood may be detected in fluids in proximity to the disease site [63]. CSF is continuous with the CNS extracellular compartment and is also the major route for seeding of metastases of malignant tumors, such as pediatric medulloblastomas, or Ewing sarcoma. As such, CSF can be representative of cancers arising in the CNS as intracranial pathology can alter the CSF proteome and therefore represents a suitable avenue to access relevant molecular information in the context of CNS disease. Besides routine cytological examination of CSF for identifying tumor cells, other biomarker studies by using CSF may contribute new information for current clinical medicine [3]. In animal models, it has been demonstrated that the CSF protein profile can be inform the presence of intracranial malignancy prior to either imaging or symptomatic detection of brain tumors [66]. This finding suggests that the CSF proteome can be altered even at the earliest stages of CNS malignancy [3]. Due to the intimate relation of CNS and CSF, CSF, as an extension of CNS, is a suitable medium for clinical biomarkers, especially for those CNS tumor-related biomarkers. By using CSF biomarkers to diagnosis and monitoring the brain and spinal cord tumors is a new and exciting field. The data collected so far supports this idea but obviously, long term studies in the future with larger data base is necessary to validation of these tests before clinical use.

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