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SNI: Neuro-Oncology

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Review Article

Liquid biopsy and tumor DNA/RNA detection in the cerebrospinal fluid of patients diagnosed with central nervous system glioma – A review article

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Received : 16 January 2023
Accepted : 11 May 2023
Published : 26 May 2023

DOI
10.25259/SNI_52_2023

Quick Response Code:

ABSTRACT

Background: Gliomas are the most common primary malignant neoplasms of the central nervous system and their characteristic genetic heterogeneity implies in a prominent complexity in their management. The definition of the genetic/molecular profile of gliomas is currently essential for the classification of the disease, prognosis, choice of treatment, and it is still dependent on surgical biopsies, which in many cases become unfeasible. Liquid biopsy with detection and analysis of biomarkers such as deoxyribonucleic acid (DNA) and ribonucleic acid (RNA) from the tumor and circulating in the bloodstream or cerebrospinal fluid (CSF) has emerged as a minimally invasive alternative to aid in diagnosis, follow-up, and response to treatment of gliomas.

Methods: Through a systematic search in the PubMed MEDLINE, Cochrane Library, and Embase databases, we reviewed the evidence on the use of liquid biopsy to detect tumor DNA/RNA in the CSF of patients diagnosed with central nervous system gliomas.

Results: After a systematic review applying all inclusion and exclusion criteria, as well as a double review by independent authors, 14 studies specifically addressing the detection of tumor DNA/RNA in the CSF of patients diagnosed with central nervous system glioma were selected in the final analysis.

Conclusion: Sensitivity and specificity of liquid biopsy in CSF are still very variable depending on factors such as the diagnostic method, collection timing, biomarker (DNA and RNA), tumor type, extension and volume of the tumor, collection method, and contiguity from neoplasm to CSF. Despite the technical limitations that still exist and prevent the routine and validated use of liquid biopsy in CSF, the growing number of studies around the world is increasingly improving this technic, resulting in promising prospects for its use in diagnosis, evolutionary follow-up, and response to the treatment of complex diseases such as central nervous system gliomas.

Keywords: Cerebrospinal fluid, Circulating tumor DNA, Circulating Tumor RNA, Gliomas, Liquid biopsy

INTRODUCTION

Central nervous system gliomas represent a complex and heterogeneous disease with multiple variants that affect from the pediatric age group to seniors. They represent about 25.1% of all primary tumors in the central nervous system and 80.8% of malignant brain tumors[25] with an

annual incidence in the United States of America (USA) of about 6.57 cases/100,000 inhabitants.[16] Glioblastoma is the most malignant variant and accounts for the most cases with an incidence of about 3.20/100,000 population in the USA[4] and an average survival of only 14.6 months.[14,37] Knowledge of the genetic profile of gliomas has become decisive for the diagnosis, treatment, prognosis, and evolutionary follow-up of this disease. New approaches such as liquid biopsy, through which different biomarkers are identified such as tumor cells, deoxyribonucleic acid (DNA) fragments, ribonucleic acid (RNA), extracellular vesicles (EVs) or proteins from the neoplasm and present in biological fluids as bloodstream, cerebrospinal fluid (CSF), or urine,[32] evolve as important tools in the management of patients diagnosed with gliomas.

In this review, through a systematic search in *PubMed* databases *MEDLINE, Cochrane Library, and Embase*, we reviewed evidence from studies that address the application of liquid biopsy in the detection of tumor circulating DNA or RNA in the CSF of patients diagnosed with gliomas of the central nervous system.

Objectives

The aim of the study was to assess the evidence on the effectiveness of liquid biopsy and detection of tumor DNA/RNA in the CSF of patients with central nervous system gliomas.

MATERIALS AND METHODS

Literature search strategy

The search was performed on *PubMed MEDLINE, Cochrane Library, and Embase*. The descriptors used in the formulation of the search strategy were defined based on the *DECS/MESH* structured health vocabulary and systematized to increase the sensitivity of the initial research. The descriptors used were: "Liquid biopsy" [MeSH Terms] AND "CSF" [MeSH Terms] AND "circulating tumor DNA/CSF" [MeSH Terms] OR "circulating microrna/CSF" [MeSH Terms] OR "cell-free DNA" [MeSH Terms] OR "tumor derived DNA" [MeSH Terms] AND "glioma" [MeSH Terms] OR "glioma/CSF" [MeSH Terms].

We do not set limits for the start date of publications, while to the final date, we established the limitation until October, 2021. After the initial research, two reviewers chose the relevant publications for the review based on the titles and *abstracts* found. Then, the full texts of the selected publications were reviewed to determine those compatible with the inclusion and exclusion criteria. In addition, we examined the references of the selected studies to verify the existence of other studies compatible with the search strategy but which were not included in the initial research.

Inclusion criteria

Studies were selected according to the following inclusion criteria: studies with liquid biopsy in CSF, patients diagnosed with central nervous system gliomas, and studies in English language.

Exclusion criteria

Studies were selected according to the following exclusion criteria: studies without availability of *abstract*, studies with other types of tumors, systematic reviews, meta-analyses, editorials, studies with exclusive blood plasma analysis, and animal studies.

RESULTS

Figure 1 illustrates the systematic approach employed to select the studies in this review based on the search strategy applied to the *PubMed/MEDLINE, Cochrane Library*, and *Embase* databases. The inclusion and exclusion criteria were applied, followed by a double review by the authors. Table 1 presents the studies included in the final phase of this systematic review.

DISCUSSION

Central nervous system gliomas represent complex neoplasms and mostly with a poor prognosis. The knowledge of its genetic heterogeneity has been decisive for the understanding of oncogenesis and consequently for the development of new treatment options and survival improvement. Liquid biopsy of CSF has emerged as a promising tool in the management of gliomas, providing information that helps in the diagnosis, definition of the genetic profile of the disease, and response to treatment.

In the pediatric subgroup, the importance and utility of liquid biopsy are highlighted due to the higher prevalence of midline gliomas, especially of the brain stem as these neoplastic subtypes usually imply difficulties for surgical biopsy, with limited treatment options and dismal prognosis.[2] About 50–80% of these tumors carry mutations in the genes encoding histone 3, more specifically HIST1H3B (H3.1K27M) and H3F3A (H3.3K27M).[9,15,23,26,35] The discovery of such mutations brings hope for the development of new targeted therapies such as *Impridone ONCO201* and *IDO1* enzyme inhibitor which in preclinical trials have demonstrated activity against mutated H3K27M gliomas.[35] Through liquid biopsy of tumor DNA in CSF, we could diagnose such mutations, define the appropriate target therapies, and monitor the therapeutic response with the option of multiple throughout the disease course.

Although there are still many limitations and challenges, in recent years, the amount of research dedicated to the study

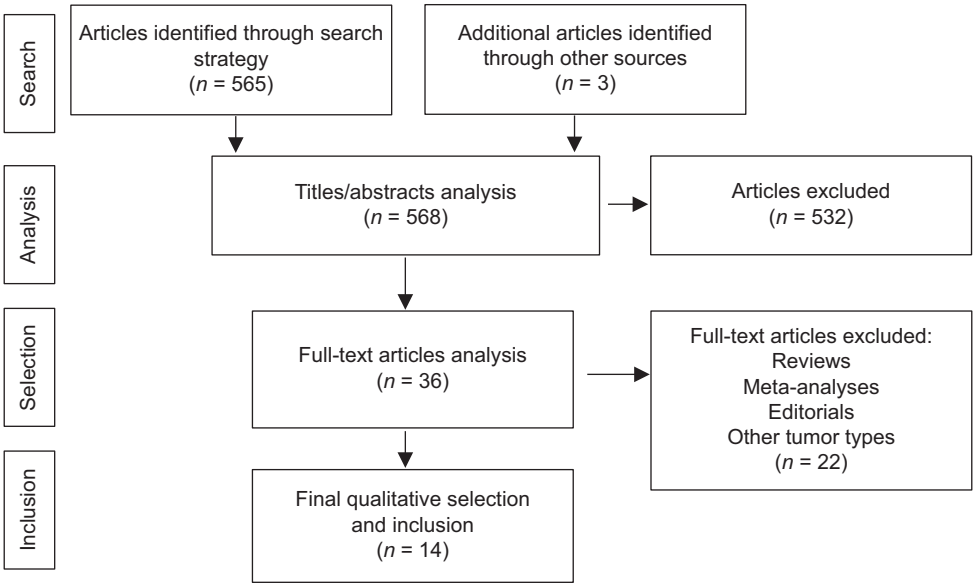

Figure 1: Articles selection flowchart. *n*: Number.

Table 1: Articles included in the final phase of the review.

Number	Title	Authors	Source	Year
1	Identification of microRNAs in the CSF as biomarker for the diagnosis of glioma	Baraniskin *et al.*[3]	Neuro-Oncology	2012
2	MiR-21 in the EVs of CSF: a platform for glioblastoma biomarker development.	Akers *et al.*	PLoS One	2013
3	Assessment of circulating tumor DNA in CSF by whole exome sequencing to detect genomic alterations of glioblastoma	Duan *et al.*	Chinese Medical Journal	2020
4	Standardization of the liquid biopsy for pediatric diffuse midline glioma using ddPCR	Li *et al.*	Scientific Reports	2021
5	Applications of CSF circulating tumor DNA in the diagnosis of gliomas.	Zhao *et al.*	Japanese Journal of Clinical Oncology	2020
6	Detection of Histone H3 mutations in CSF-derived tumor DNA from children with diffuse midline glioma	Huang *et al.*	Acta Neuropathologica Communications	2017
7	Clinically relevant and minimally invasive tumor surveillance in pediatric gliomas using liquid biome	Panditharatna *et al.*[27]	Clinical Cancer Research	2018
8	Targeting and therapeutic monitoring of H3K27M-mutant glioma	Wierzbicki *et al.*	Current Oncology Reports	2020
9	Detection of cell-free DNA fragmentation and copy number alterations in CSF from glioma patients	Mouliere *et al.*[21]	EMBO Molecular Medicine	2018
10	Molecular profiling of tumors of the brainstem by sequencing of CSF-derived circulating tumor DNA	Pan *et al.*	Acta Neuropathologica	2019
11	Low detection rate of H3K27M mutations in CSF obtained from lumbar puncture in newly diagnosed diffuse midline gliomas	On *et al.*	Diagnostics	2021
12	Tracking tumor evolution in glioma through liquid biopsies of CSF	Miller *et al.*	Nature	2019
13	A novel high-sensitivity assay to detect a small fraction of mutant IDH1 using ddPCR	Hirano *et al.*[13]	Brain Tumor Pathology	2018
14	PCR-detection of tumor-derived p53 DNA in CSF.	Harker Rhodes *et al.*[29]	American Journal of Clinical Pathology	1995

Evs: Extracellular vesicles, CSF: Cerebrospinal fluid, ddPCR: Droplet digital polymerase chain reaction, DNA: Deoxyribonucleic acid, RNA: Ribonucleic acid, MiR-21: Micro-Ribonucleic acid-21, IDH1: Isocitrate dehydrogenase 1

of tumor biomarkers present in the CSF and improvement of diagnostic techniques has grown dramatically. It is known that tumor DNA/RNA is difficult to detect in CSF due to its fragmentation into small chains, short half-life,[6] and its low amount in CSF, ranging from 0.0003 to 3.76 ng/μL.[15] Even so, preliminary results demonstrate CSF advantages over blood in the analysis of tumor DNA/RNA.[7,9,32] As demonstrated in the study by De Mattos-Arruda *et al*., multivariate analyzes involving the comparison of the genetic profile of the tumor and the circulating DNA, as well as changes in the genetic profile of the neoplasm, were identified with greater sensitivity and accuracy in CSF than in plasma.[7]

In our review, we found that the diagnostic sensitivity of liquid biopsy in CSF is still very variable depending on factors such as the diagnostic method, collection timing, biomarker (DNA, RNA), tumor type, extension and volume of the tumor, collection method, and contiguity from neoplasm to CSF.[15,23] In the study by Pan *et al*., using next-generation sequencing techniques (NGS) to compare the genetic profile identified in the tumor and in the circulating tumor DNA, they reached a sensitivity of 97.3%[26] while in the study by Huang *et al*. using the Sanger technique, the sensitivity for detection of histone H3 mutations in the CSF was 87.5% with a specificity of 100%.[15] Higher concentrations of tumor DNA were found in those patients with a large tumor volume or in those with intraventricular tumor extension or in the vicinity of the ventricle.[10,15,20] On the other hand, studies using lumbar puncture to collect CSF resulted in a lower sensitivity for detection of tumor DNA when compared to the levels detected in samples collected through ventricular shunts.[23] Given the variety of diagnostic methods (Sanger, ddPCR, and NGS), biomarkers and collection methods used in the studies of this review, the need to standardize the steps for obtaining samples, analysis, and interpretation of results in liquid biopsy is evident.

Although our review focused on analyzing the use of tumor DNA/RNA, we found that other biomarkers have shown promise in the CSF liquid biopsy of patients diagnosed with gliomas. Due to the relevance of the various biomarkers researched, below we highlight some characteristics of those most used today:

Circulating tumor DNA

Small fragments of 150–200 base pairs of tumor-derived DNA, circulating in the bloodstream or CSF and not coupled to cells.[11,34] They typically originate from apoptotic or necrotic tumor cells, having a short half-life (<1.5 h) and rapid degradation when in their free form.[8]

Circulating tumor RNA

This group includes micro-RNAs, long noncoding RNAs, and small non-coding RNAs.[6] Micro-RNAs are small RNA molecules (21–24 nucleotides) that do not code for proteins but appear to play a role in communication and functional regulation in normal and tumor cells. They can be secreted coupled in EVs or in a free form (cell-free) with a longer half-life when compared to circulating tumor DNA.[12,14,34] Since the first studies describing micro-RNAs, more than 2000 types have been defined[34] and some of them, such as miR-21, seem to have a particular relevance in gliomas with important applications ranging from helping to distinguish pseudo-progression and radionecrosis to prognostic assessment and treatment response.[1,12,28,34,36]

EVs

Both tumor cells and normal cells secrete EVs that carry diverse contents including proteins, lipids, DNA, and RNA that seem to perform functions of intercellular communication and regulation.[6,34] These structures typically consist of two phospholipid layers but due to some structural differences, they can be divided into three subgroups: exosomes (30–100 nm) derived from the endosomal membrane; microvesicles (100 nm–1 μn) derived from the cell membrane, and apoptotic bodies produced in the cell death process (1–5 μm).[18,19] Together with the structures described in this review, EVs have played a prominent role in the evaluation of gliomas, with studies demonstrating their role in oncogenesis[6] as well as representing biomarkers of the evolution of diseases such as gliomas of the central nervous system.[6,24]

Circulating tumor cells

Cells derived from the primary tumor and that enter the bloodstream or CSF are called circulating tumor cells.[6] These cells can be isolated or organized in "clusters" and be precursors of brain metastases. It is postulated that their scarcity in biological fluids is a consequence of some factors such as the blood-brain barrier or in cases of gliomas, that tumor cells demand tumor growth factors and specific microenvironments that are absent outside the central nervous system.[6,22]

Proteins

Genetic alterations in neoplasms such as gliomas alter the expression of cellular proteins and consequently define specific profiles that can be used as tumor biomarkers for diagnostic, therapeutic, and prognostic purposes.[17,31,33] Due to this promising potential in the management of gliomas, recent studies combining advanced laboratory analysis techniques and bioinformatics have identified proteins in the CSF such as IL6, HSPA4, and WNT4 E that seems to play an important role in the pathogenesis of these neoplasms.[5,30,31]

CONCLUSION

Through this review, we investigated the effectiveness of liquid biopsy and detection of tumor DNA/RNA in the CSF of patients diagnosed with gliomas of the central nervous system. The diagnostic sensitivity and specificity of liquid biopsy in CSF are still very variable depending on factors such as the diagnostic method, collection timing, biomarker (DNA and RNA), tumor type, extension and volume of the tumor, collection method, and contiguity from neoplasm to CSF. Despite the technical limitations that still exist and prevent the validated and routine use of liquid biopsy in CSF, the growing number of studies around the world increasingly improves it, resulting in promising perspectives for its use in diagnosis, evolutionary follow-up, and response to the treatment of complex diseases such as central nervous system gliomas.

Declaration of patient consent

Patient's consent not required as there are no patients in this study.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

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How to cite this article: Borba LA, Passos G, Oliveira I. Liquid biopsy and tumor DNA/RNA detection in the cerebrospinal fluid of patients diagnosed with central nervous system glioma – A review article. Surg Neurol Int 2023;14:183.