NARRATIVE REVIEW

Serum liquid biopsy for brain tumours: a scoping analysis of practicable approaches in low- and middle-income countries

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Abstract

Approaches to brain tumour diagnosis and detecting recurrence after treatment are costly and significantly invasive. Developing peripheral-sample liquid biopsy tools is the key to enhancing our ability to prognosticate brain tumour subtypes and molecular heterogeneity. The present scoping review was designed to discuss current updates in liquid biopsy tools for diagnosis and guiding clinical management of brain tumours; we evaluated the literature within the context of low-and-middle-income country challenges. Circulating tumour cells (CTCs), circulating tumour DNA (ctDNA), cell-free DNA (cfDNA), extracellular vesicle-associated biomarkers, protein biomarkers, microRNAs, and serum metabolites are discussed with the collation of current data supporting their utility in liquid biopsy. Further challenges to implanting liquid biopsy tools at a systematic level are highlighted.

Keywords: Tumour, DNA, Neoplastic Cells, Liquid Biopsy, Brain Neoplasms, MicroRNAs, Extracellular Vesicles

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Introduction

Brain tumours are a burgeoning healthcare issue within low-and-middle-income countries (LMICs) due to costs associated with treatment, response assessment, and follow-ups.[1] These tumours are genetically heterogenous and require precise molecular characterisation for targeted therapy. Many resource-constrained healthcare systems cannot afford to provide adequate and continued care to patients, many of whom live in the global south. Data from South Asia shows many patients abandon post-surgical chemotherapy and radiation treatment and are lost-to-follow-up, adding to the overall morbidity and mortality.[2-4] A significant barrier to follow-up is the considerable distance from neurosurgical access within LMICs; a recent study from Pakistan indicated that 59% of patients had to travel over 50 km for neuro-oncological surgery, with 15.7% travelling over 500 km.[4] Radiological imaging can be used for diagnosing based on tumour location, morphology, and signal intensity; however, there is limited information about molecular features, which are essential for treatment. Currently, molecular characteristics can be identified after surgery based on brain tissue which can prolong and significantly impact the initiation of adjuvant treatment.

Moreover, postoperative radiology cannot definitively quantify tumour recurrence and differentiate it from pseudo-progression.[5,6] The high cost of imaging results in treatment delays in many parts of the world.[7] The brain is a difficult-to-access organ for tissue diagnosis; repeat biopsies and molecular assessment of the evolving tumour and peri-tumoral region are not feasible.

Through the detection of materials shed by tumours, including circulating tumour cells (CTCs) and genomic specimens in bio-fluids (e.g., blood and cerebrospinal fluid), we can identify biomarkers that reflect tumour microenvironment and dynamic evolution in real-time.[8] The high specificity and sensitivity of this liquid biopsy (LB) can potentially reduce the financial burden and aid the expanding the role of molecular characterisation in prognosticating brain tumours. LB is a robust and minimally invasive screening tool allowing early tumour detection and reliable follow-up, whether this assessed treatment response, recurrence, or evolution of tumour biology. In CNS neoplasms, CSF is a source of molecular markers and can be used to track the evolution of gliomas.[9,10] However, the procedure to obtain CSF is invasive and risky in patients harbouring CNS tumours, which limits its use for serial assessment of the disease. In contrast, a liquid blood biopsy is minimally invasive, quick, and can be performed longitudinally within low-cost collection centres worldwide.

The use of blood-derived liquid biopsy is limited by the

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minimal concentration of CNS tumour products due to the presence of the blood-brain barrier. Moreover, storage, and shipping can compromise the integrity of the biomolecule or biomarker, producing varying results. For example, the Ethylenediaminetetraacetate (EDTA) used to maintain blood longevity may cause genomic DNA contamination if stored for a long time. Due to prevalent issues in LMICs, such as poor transport facilities and limited standardized diagnostic facilities which can run genetic tests, the sample is unlikely to reach the diagnostic facility in good condition.

This review highlights the utility of liquid biopsy in diagnosing and guiding brain tumour treatment through a systematic review with scoping synthesis. We discuss the practicality from the standpoint of LMICs with the suggestion for further implementation.

Methodology

To explore potential challenges in implementation of liquid biopsy for brain tumours, we aimed to conduct a scoping review of current literature with relevant exploration of current methods as well. This allows us to provide updated evidence on multiple facets of LB in brain tumours while exploring the systemic issues with implementation. A systematic literature search was conducted on 18/11/2022 using permutations of keywords, and similar terms, including "liquid biopsy", "brain tumour" and "neuro-oncology", using Scopus and PubMed (MEDLINE) databases – after removal of duplicates, a total of 706 articles were identified for title and abstract screening. This process was conducted in duplicate, leading to the inclusion of 46 articles for the present scoping review. Articles in non-English languages, reviews, other systematic review and meta-analyses, and articles that did not specify method of liquid biopsy used were not included in the review. Only data collected from human subjects was included. Data were collated and an expert review was made regarding utility and challenges faced by scientists in LMICs for implementing LB in neuro-oncology by the senior author.

Liquid biopsy in the management of brain tumours

Genetic profile studies of tumour tissues have led to the discovery of biomarkers specific to tumour types. These include circulating tumour cells (CTCs), circulating tumour DNA (ctDNA), cell-free DNA (cfDNA), circulating proteins, extracellular vesicles and exosomes, long non-coding RNA (lncRNA), micro-RNA (miRNA) and other genetic alterations. LB methods intend to detect these genetic markers in peripheral bio-fluids to provide an alternative to the current gold standard of tissue biopsy and capture the heterogeneity within the tumour.[11] Collated results are presented in Tables 1-3, discussing study design and outcomes presented.

Circulating tumour cells

Serial tumour cells are routinely detected in CSF cytology for staging certain brain tumour types. Circulating tumour cells in the body fluids might indicate tumour metastasis and may be helpful in diagnosis, progression, and prognosis.[12,13] An essential advantage of CTCs over cfDNA or ctDNA is their longer half-life ranging from 1 - 2.4 hours if taken from the patient's blood or CSF.[14] Besides pre-analytical pitfalls in handling the sample of body fluids, as described later, CTCs are present in low concentrations in the liquid biopsy samples, especially in early-stage tumours.[15] For example, in one study, only 29 (20%) of the cohort, including 141 GBM, had detectable CTCs, and in many cases, only one cell was found per 10 mL of blood per patient.[16] The discovery of new devices, such as the Cell Collector from GILUPI (GMBH, Potsdam, Germany), enables the isolation of circulating tumour cells directly from the arm vein of the patient may increase the concentration of CTCs achieved.[17]

Ctdna and Cfdna

DNA is released from cells into body fluids by physiological and pathological cell death and is called (cfDNA).[18,19] Parts of cfDNA derived from the tumour cell are called circulating tumour DNA (ctDNA). The size of ctDNA ranges from 70 to 200 base pairs, a longer DNA fragment than normal cfDNA. They are also released randomly in micro-vesicles or exosomes and carried to other cells. Bagley et al. reported that cfDNA could be a prognostic tool in GBM patients and later confirmed it in another study with a larger independent patient cohort.[20,21]

Moreover, it has also been determined that an increase in cfDNA concentration from before surgery to post-adjuvant treatment is associated with worse progression-free survival (PFS) and overall survival (OS), suggesting that cfDNA dynamics during the treatment phase may have a role in determining therapeutic response in GBM.[21] García-Romero et al. isolated ctDNA, including EV-derived DNA, from 29 paediatric patients diagnosed with CNS tumours from serum, plasma, and CSF and showed that ctDNA found in serum and plasma could determine genetic characteristics of the tumour. They identified BRAF V600E mutations in both liquid biopsy sources.[22]

Diffuse midline gliomas are one of the most lethal due to their location, making it difficult to obtain tissue samples for biopsy with significant morbidity associated with surgical intervention. Cantor et al. demonstrated

Table 1: Studies regarding microRNA use as liquid biopsy tools.

First Author	Publish Year	Cancer Type	Controls	Patients / Controls	miRNAs	Detected Sample	Method Used	Summary
Hallal S. et al.[47]	2020	GBM	Astrocytoma II-III	12/5	miR-486-3pmiR-106b-3pmiR-378a-3p miR-21-5p miR-146b-5p miR-196b-5p miR-574-5p miR-222-3p miR-16-2-3p miR-193a-5p miR-5100 miR-335-5p (↑) miR-1306-3p ((↓)	CUSA-EVs	NGS	Functional pathway analysis of mRNAs targeted by CUSA-EV miRNAs (212 species DE in GBM relative to GII-III; $p \leq 0.05$) shows clear associations to the GBM signaling pathway and more broadly, 'molecular mechanisms of cancer.'
Iannó M. F. et al.[30]	2022	Diffuse Intrinsic Pontine Glioma		47	miR-4714-3p miR-551b miR-4505 miR-6090 miR-6089 miR-3960 miR-936 miR-1207-5p miR-202-3p miR-3676-5p miR-4634 miR-4539 miR-4299 ((↑)	Serum		This study provides Class II evidence that a signature based on 13 circulating miRNAs is associated with the risk of disease progression.
Morokoff A. et al.[31]	2020	Glioma	Healthy controls	91/17	miR320e miR-223 miR-16-5p miR-484 miR520a miR-532 miR-630 miR651 miR-761	Serum exosomes and non-exosomes	ddPCR	This study reports the first systematic longitudinal study of serum microRNA biomarkers in glioma, finding that levels of miR-320e, miR-223 and miR-21 are correlated with tumor burden based on MRI.
Olioso D. et al.[32]	2021	GBM, Anaplastic Astrocytoma		57	miR-21 miR-222 miR-124-3p ((↑)	Serum exosomes		No correlation found between exosomal miRNA expression and PFS or OS at the beginning of the study or after RT and first cycle of TMZ. Data reported in the study shows that exosomal miRNAs may predict response to therapy during follow-up in HGG patients.
Sippl C. et al.[33]	2022	GBM	Healthy Controls	60/30	miRNA-181d (↑)	Plasma	RT-qPCR	miRNA-181d plasma expressions has no significant impact on

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								survival or prognosis. However, some previous work shows that it has an impact on survival if local carmustine wafer therapy is applied.(48)
Swellam M. et al.[34]	2021	GBM	Healthy controls	25/20	miR-17-5p miR-125b miR-221 (↑)	Serum	RT-qPCR	An increase in serum miR-17-5p, miR-125b, and miR-221 levels in GBM patients is related to worse progression-free survival and overall survival.
Tomeva E. et al.[49]	2021	Brain cancer	Healthy controls (n=15), non-brain cancers (n=188)	9/203	miR-133a-3p miR-23a-3p ((↑)	Serum	RT-qPCR	This study analyzed cell-free DNA (cfDNA) mutations and methylation, as well as circulating miRNAs (miRNAs) in plasma samples from 97 patients with cancer (bladder, brain, breast, colorectal, lung, ovarian, pancreas, prostate, stomach) and healthy controls.
Zhang H. et al. [50]	2019	GBM	Healthy controls	95/60	miRNA-100 (↑)	Serum	RT-qPCR	miR-100 expression levels is down-regulated in GBM compared to the healthy controls and closely correlated with shorter survival. Their levels is significantly increased following treatment indicating sensitivity to treatment response.

Table-2: EV as liquid biopsy.

First Author	Publish Year	Cancer Type	Patients	Control group	EV-associated biomarker	Alteration	Biological Sample	Methods of biomarker discovery	EV isolation Method	Summary
Batool SM. et al.[25]	2022	GBM	54	Healthy controls; EGFR wt patients	EGFRvIII mRNA		Plasma	ddPCR	ExoRNeasy	Establishes a sensitive plasma-based droplet digital PCR (ddPCR) assay for the detection of the EGFRvIII mutation in EV-derived RNA.
Bukva M. et al.[51]	2021	GBM	46	Lumber disc herniation patients; Other brain	Raman measurements		Serum	Raman spectroscopy	Differential Centrifugation	Results support that Raman spectroscopic

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				tumors						analysis of circulating sEV-enriched isolates is a promising method that could be used in the diagnosis of CNS tumors.
Cilibrasi C. et al. [52]	2022	GBM	15	Healthy controls	VWF; C3; FCGBP; PROS1; SERPINA1	Overexpression	Plasma	Mass spectrometry	Differential Ultracentrifugation	Describes an inflammatory biomarker signature was in sEVs from GB patients.
Garcia L. et al.[53]	2019	GBM	19	Healthy controls	IFN-γ; IL-10; IL-3; CD80, CD86; ICOSL	Underexpression	Plasma	Cytokine assay, ELISA	Density gradient Ultracentrifugation	Highlights differences in size and frequency in plasma EVs between GBM patients and normal donors, and presents evidence for decreased expression of inflammatory markers in GBM patients' exosomes.
Ebrahimkhani S. et al. [54]	2018	GBM	12	Healthy Controls; Grade II-III glioma	miR-182-5p; miR-328-3p; miR-339-5p; miR-340-5p; miR-485-3p; miR-486-5p; miR-543	Differential expression	Serum	smallRNA sequencing	Size exclusion chromatography	Identifies distinct and superior miRNA signatures from previously reported "free-circulating" miRNA studies in GBM patients

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Dobra G. et al.[55]	2020	GBM	24	Lumber disc herniation patients; Other brain tumors	PF4; S100A14; HSPA8; HBG1; CASP14; HSPB1; CCCT; SBSN; S100A7; FLG2; IGLL1; SPRR2E; ANPEP; MMP9; FN1; FGB; MMRN1	Differential expression	Serum	LC-MS	Ultracentrifugation	Shows that even a low-efficacy sEV enrichment method may be appropriate to enhance the analytical applicability of serum samples for CNS cancer monitoring
García-Romero N. et al. [56]	2017	Glioma	21		IDH1G395A gDNA	Overexpression	Plasma; Serum	Fast Cold-PCR	Differential Ultracentrifugation	Supports the idea that EVs secreted by brain tumor cells can cross the BBB, whether intact or disrupted, and enters the bloodstream. Therefore, the analysis of their cargo might be useful as a biomarker.
Graziano F. et al. [57]	2021	Glioma; Meningioma	34	Healthy controls	Hsp60; miR-1; miR-206; miR-663	Differential expression	Plasma	qRT-PCR	Differential Ultracentrifugation	Reveals that EVs with Hsp60 and related miRNAs increase in patients' blood in a manner that reflects disease status
Hallal S. et al.[58]	2020	Glioma	41	Healthy controls	AIDA; ARHGEF10; BNIP3L; FYB1; KMT2D; MAP7; MAST4;	Differential expression	Plasma	SWATH-MS; LC-MS/MS	qEV	Highlights the potential for SWATH-MS to define circulating-EV biomarkers

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					PDE8A; POLR2D; RENBP; SLC25A17; CDC40; TPST2CETN3; PPP1R11; SYT7					for objective blood-based measurements of glioma activity using plasma-EV derived proteome profiles.
Chandran I. et al. [59]	2019	GBM	65	Low Grade Glioma	SDC1	Overexpression	Plasma	LC-MS/MS; ELISA	Size exclusion chromatography	Identifies SDC1 as a plasma derived-EV constituent for noninvasive differentiation between GBM and LGG
Jones P. et al.[60]	2019	GBM	6		PpIX	Overexpression	Plasma	Imaging flow cytometry	ExoEasy	Highlights the potential of plasma-derived PpIX-positive EV using IFC based diagnostics for malignant gliomas.
Li P. et al. [61]	2022	High Grade Astro-cytoma	30	Healthy controls	circ-0075828; circ-0002976; circ-0003828	Expression	Serum	circRNA Sequencing (qRT-PCR)		Shows that the serum exosome circRNA is potentially useful for HGA liquid biopsy and prognosis monitoring.
Maas S. et al.[42]	2020	GBM	30	Healthy controls	PpIX	Expression	Plasma	High-resolution flow cytometry	Ultracentrifugation	Assesses whether PpIX accumulates in GB-derived EVs and whether these EVs could be isolated and characterized to enable a

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										liquid biopsy in GBM
Piazza A. et al.[62]	2022	Glioma	14	Non-specific cephalea patients	exoDNA levels	Variation	Plasma	Fluorometry	ExoEasy	Shows the variation of the concentration of exoDNA in the plasma across the different stages of tumor growth
Ricklefs F. et al. [63]	2020	Glioma	29	Healthy controls	FASN	Overexpression	Plasma	Imaging flow cytometry	Ultracentrifugation	Shows that combined marker profiling is more sensitive at detecting subtle shifts in EV subpopulations than considering only single markers and that elevated FASN+/CD63+ as well as FASN+/CD81+ EVs are characteristic of glioma patients
Rosa P. et al.[64]	2022	GBM	26	Non-specific cephalgia patients	NF1; exoDNA levels	Mutation; Decreased levels of exoDNA	Plasma	NGS	ExoEasy	Demonstrates the technical feasibility of the mutational analysis of the genomic cargo in circulating EVs
Wang H. et al.[65]	2019	Glioma	23	Healthy controls	EGFR; PTTG1; NLGN3	Overexpression	Serum	Flow cytometry; qRT-PCR	Ultracentrifugation	Shows the potential of EGFR, NLGN3 and PTTG1 in EVs for

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										detecting glioma using optimized flow cytometry.

Table 3: cfDNA as liquid biopsy.

First Author	Publish Year	Cancer Type	Patients	Detected Alteration	Frequency of detection	Sample Used	Methods	Sensitivity	Specificity	Summary
Bagley S. et al. [66]	2021	GBM	62	cfDNA levels		Plasma	qPCR			Increased cfDNA concentration from pre-operative to post-chemoradiotherapy is associated with worse subsequent PFS and OS, suggesting that on-therapy cfDNA dynamics may have a role in assessing therapeutic response in patients with GBM.
Cantor E. et al.[24]	2022	DMG	28	H3.3K27M mutation	53/62 (plasma samples); 28/29 (CSF samples)	Plasma; CSF	ddPCR	85.4% (plasma); 96,5% (CSF)		Patterns of change in H3K27M VAF over time demonstrate clinical utility in terms of predicting progression and sustained response and possible differentiation of pseudoprogression and pseudo-response.

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Fontanillies M. et al.[67]	2020	GBM, Gliosarcoma	52	TERT promoter mutation	2 out of 46 patients	Plasma	ddPCR			In contrast to ctDNA using TERTp mutation detection, the cfDNA concentration varies significantly over the course of treatment and may be a biomarker of PD during the TMZ phase
Garcia-Romero N. et al. [23]	2019	Pediatric CNS tumors	29	BRAF V600E	29/29	Serum;Plasma ; CSF	ddPCR	25% (plasma); 50% (serum)	77.8% (plasma); 100% (serum)	The first study to compare the different sources of liquid biopsies in pediatric cancers
Husain A. et al. [68]	2022	ADG	50	cfDNA levels		Serum	qPCR			High baseline cfDNA concentration predicted worse treatment response independent of other prognostic factors. The study also compares the pre-operative and postoperative serum cfDNA mutations in matched tissue from ADG patients
Izquierdo E. et al. [69]	2021	HGG; DMG	27	H3F3A, IDH1, BRAF, ACVR1, PI3KCA mutations;	7/27 (plasma samples); 6/9 (CSF samples); 2/6 (serum	Plasma; Serum; CSF; Cystic Fluid	ddPCR	10%		This study describes the validation of a number of

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				MYCN amplification	samples); 1/1 (cystic fluid sample)					ddPCR assays for the detection of point mutations in cfDNA.
Kang K. et al.[70]	2021	Glioma; CNS metastasis	9	BRAF V600E	4 out of 5 patients	Plasma	ddPCR	80%	100%	Demonstrate technical development a droplet digital PCR assay for the detection of BRAF V600E in plasma of patients with primary and metastatic brain tumors
Koga T. et al.[71]	2022	GBM	29	EGFRvIII breakpoints		Plasma; CSF	qPCR; PCR mapping; sWGS			EGFRvIII-derived PCR amplicons were not obtained from either cfDNA or evDNA derived from plasma, even with modifications to enhance sensitivity, such as preamplification and heparinase I treatment.
Li D. et al.[72]	2021	DMG		H3.3K27M mutation		CSF; Plasma	ddPCR			Employs vacuum-concentration of pre-amplified ctDNA, which increased test sensitivity without decreasing specificity), enabling

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										target mutation detection in patient-matched tumor tissue, CSF and blood specimens
Mouliere F. et al. [73]	2021	Glioma	35	Mutation in multiple genes; cfDNA fragmentation	10/16 (urine sample); 10/12 (plasma samples); 7/8 (CSF samples)	CSF; Plasma; Urine	patient-specific hybrid-capture panels; sWGS	59%	92%	Reveals possible difference in the fragment sizes of urine cftDNA in cancer patients as compared to healthy individuals
Nassiri F. et al.[74]	2021	Intracranial tumors	220	Glioma-specific DNA methylation-based signatures		Plasma	Whole genome cfMeDIP-seq			Distinguishes gliomas from intracranial metastasis and healthy controls without relying on information obtained from a tumor tissue biopsy
Muralidharan K. et al.[75]	2021	Glioma	157	TERT promoter mutation	33 out of 46 plasma samples	Plasma	ddPCR	62.50%	90%	Demonstrates a TERT promoter mutation assay utilizing high affinity LNA enhanced probes and an additive 7dG for detection and monitoring of the mutations in tumor tissue and cfDNA of matched

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								plasma of patients
Nørøxe D. et al. [76]	2019	Glioma	8	cfDNA levels		Plasma	Fluorimetry	Shows good tendency between cfDNA and treatment course and -response, respectively with the highest levels at progression.
Okamura R. et al. [77]	2020	Glioma	135	CH-associated genes: ATM, BRAF, BRCA1, EGFR, FBXW7, GNAS, IDH1, JAK2, MET, NF1, PDGFRA, and TP53	29/135 (characterized alteration); 27/135 (VUSs in cfDNA)	Plasma	NGS	CH-type cfDNA mutations is an independent prognostic factor for shorter survival
Pages M. et al. [78]	2022	Pediatric Brain tumors	258	Copy number alteration and mutation in multiple genes	78/132 (at least one CNA at a chromosome arm level)	Blood; Urine; CSF	ULP-WGS	Systematically evaluates the feasibility of profiling pediatric brain tumours using ctDNA obtained various biomes. Most samples had insufficient somatic mutations discoverable by the sequencing panel to provide sufficient power to detect tumor fractions.

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Palande V. et al. [41]	2022	GBM	25	cfDNA levels; TP53, EGFR, NF1, LRP1B, IRS4 mutations; KDR-PDGFRA, NCDN-PDGFRA, COL1A1–PDGFB, NIN–PDGFRB, FGFR1–BCR, CEP85L–ROS1 and GOPC–ROS1 gene-gene fusions		Blood	Fluorimetry; NGS	80%	95%	Suggests that integrated analysis of cfDNA plasma concentration, gene mutations, and gene–gene fusions can serve as a diagnostic modality for distinguishing GBM patients who may benefit from targetted therapy.
Panditharatna E. et al.[79]	2018	pDMG	48	H3.3K27M mutation	20/23 subjects (CSF samples) ; 18/20 subjects (plasma samples)	Plasma; CSF	ddPCR	88%		Shows that CSF and plasma ctDNA analysis of children with DMG is feasible, shows promise for detecting mutational load and provides an additional means for molecular disease characterizati on
Piccioni D. et al. [80]	2019	Primary Brain Tumors	419	Copy number alteration, Variant allele fraction and mutation of multiple genes	211/ 419 patients	Plasma	NGS	55%		Contrary to other cfDNA studies which postulated that ctDNA would not cross the BBB, this study found that half of the patients with primary brain

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										tumours had detectable cfDNA alterations with 48.9% of these having a potentially genomically targetable alteration
Sabedot T. et al.[81]	2021	Glioma	149	Glioma-specific DNA methylation-based signatures		Serum	Genome-wide Methylation array	100%	97.78%	Developed and verified a score metric (the "glioma-epigenetic liquid biopsy score" or GeLB) that optimally distinguished patients with or without glioma
Szadkowska P. et al.[40]	2022	Brain Tumors	84	Mutation in multiple genes; Copy number alteration	8/84 patients (tumor specific alteration); 32/84 patients (potentially pathogenic alteration)	Plasma	NGS			Shows that slight improvements in isolation, library preparation, and mutational analyses of ccfDNA lead to better detection of tumour-specific genetic alterations
Tuna G. et al.[82]	2022	Glioma	49	IDH1 R123H mutation	12/19 patients (CSF samples); 1/4 patients (plasma samples)	CSF; Plasma	ddPCR			Provides evidence that the analysis of CSF ctDNA may complement the diagnosis of IDH1 R123H

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	mutation at a rate of 63.2%. Also shows D-2-HG concentrations in CSF can distinguish between IDH1 wild-type and mutant individuals with acceptable sensitivity and specificity.

CUSA-EV: cavitron ultrasonic surgical aspirate extracellular vesicles; NGS: next-generation sequencing; ddPCR: Droplet Digital PCR; PFS: progression-free survival; OS: overall survival; RT: radiation therapy; RT-PCR: reverse-transcription PCR; EV: extracellular vesicles; BBB: blood-brain barrier; qRT-PCR: Quantitative Reverse Transcription PCR; SWATH-MS: Sequential Window Acquisition of All Theoretical Mass Spectra; LC-MS: Liquid Chromatography Mass Spectrometry; IFC: Intraoperative flow cytometry; sWGS: shallow whole genome sequencing; ULP-WGS: ultra-low-pass whole-genome sequencing.

feasibility and clinical efficacy of serial cf-t DNA in plasma and CSF of DMG in addition to serial monitoring through imaging. The pattern of changes in VAF values over time demonstrates utility in correlating with a sustained response, predicting progression, and identifying pseudo-progression and pseudo-response,[23] including extremely low abundance in serum compared to CSF limits the use of cfDNA for liquid biopsies. Moreover, they degrade readily as compared to CTCs. Although the studies above demonstrate that cfDNA can be used to screen for brain tumours, additional investigations sometimes add to the total cost, thus making it inapplicable for use in a resource-limited setting.

Extracellular vesicles and exosomes

Exosomes are extracellular vesicles that contain cell contents such as DNA, RNA, proteins, and metabolites. They are incredibly stable outside cells and have the potential to be a perfect liquid biopsy tool. Using RNA derived from extracellular plasma vesicles, Batool et al. demonstrated the presence of EGFRvIII mutations in glioma patients. The same mutation was also detected in the tumour tissue of the patient. The sensitivity and specificity for the detected mutation in plasma compared with tumour tissue analysis were reported to be 72.8% and 97.7%, respectively.[24] In another study, Hallal S. et al. reported significant changes in miRNA and piRNA expression in GBM-EVs compared to GII-III EVs, several of which play essential roles in glioma genesis. Their results showed that CUSA-EV miRNAs could elucidate and validate potential prognostic biomarkers for GBM. A key example is the detection of IDH-1 transcripts within circulating EVs, allowing classification of glioblastoma through non-invasive methods.[25]

Indeed, there are problems with the clean filtration and isolation of exosomes. Liu et al. highlighted that serum might not be the perfect choice for a representative sampling of circulating EVs, as a high fraction of EVs may be lost during coagulation.[26] Furthermore, blood components (e.g., platelets) may release micro-vesicles (MV) during clotting, altering the original MV content of blood samples. Besides, they are incredibly adhesive to standard laboratory plastic, reducing the isolated concentration.[27]

Micrornas

MicroRNAs, small RNA molecules devoid of coding functions, circulate within the bloodstream in highly stable forms, offering potential applications in liquid biopsy.[28] Various miRNAs, notably miR-21, miR-15b, miR-125b, and miR-223, have been identified in the cerebrospinal fluid (CSF) of adult brain tumour patients. These particular markers exhibit notable specificity and sensitivity for detecting gliomas and medulloblastomas. Iannó MF. et al. outlined a distinctive profile comprising 13 circulating miRNAs, where increased expression of miR-4714-3p, miR-551b, and miR-4505 correlated with improved prognosis.[29] Conversely, higher expression of the remaining ten miRNAs in the signature (miR-6090, miR-6089, miR-3960, miR-936, miR-1207-5p, miR-202-3p,

miR-3676-5p, miR-4634, miR-4539, and miR-4299) was associated with a poorer prognosis. Markoff et al. identified a 9-gene miRNA pattern that could accurately discern between glioma and healthy individuals with a precision rate of 99.8%. Among these, miR-223 and miR-320e exhibited dynamic fluctuations closely correlating with tumour volume in low-grade gliomas (LGG) and glioblastomas (GBM), respectively. Notably, levels of miRNAs remained unchanged in instances of pseudo-progression, showcasing the potential of this test in steering treatment decisions.[30] Olisio et al. demonstrated that high-grade glioma (HGG) progression coincided with escalated expression of serum exosomal miR-21, miR-222, and miR-124-3p during postoperative monitoring.[31] In the context of GBM, Sippl et al. identified miRNA-181d as a promising molecular marker reliably detectable in blood samples.[32] Conversely, Swellam M. et al. established a correlation between increased serum levels of miR-17-5p, miR-125b, and miR-221 in GBM patients and poorer progression-free survival, as well as worse overall survival.[33]

Metabolites and proteins

Tumour-associated proteins i.e. 'onco-metabolite' accumulation may be a promising approach to evaluating the consequences of brain tumour-associated mutations. Small molecules such as 2HG (D-2-hydroxyglutarate) have been shown to accumulate in glioma with IDH-1 mutations and can be detected within serum samples. A study of 84 patients comparing 2HG with mass spectrometry showed plasma and urine levels to be correlated with treatment response and disease recurrence.[34] This can further be validated through MR spectroscopy detection of 2HG within the brain as a non-invasive radiomics marker; however, further utility may be limited within many LMIC centres.[35] Other potential proteomic targets suggested have been haptoglobin α2, YKL-40, and AHSG serum levels as strong correlates for tumour grade and prognosis.[36] ELISA and mass spectroscopy are the most commonly used methods to quantify serum proteins; ELISA is easily available and accessible in limited resources, although inferior to mass spectroscopy in protein discovery.[37]

Challenges in utilising liquid biopsy for brain tumour management in LMICs

Significant progress has been made in liquid biopsies for brain tumours; however, its application in LMICs is a considerable challenge. The methodologies employed to look for the components of tumours subject to liquid biopsy face certain obstacles that hamper their optimal functioning. Here we highlight the problems faced in the pre-analytical, analytical, and post-analytical phases of liquid biopsy processing.

Pre-analytical phase

This phase starts with collecting the bio-fluid sample and ends with isolating the component under investigation. Most errors in the sample evaluation and workflow occur in this phase and can limit the integrity of the sample and the data quality.

Due to limited equipped facilities, samples must be transported over considerable distances for analysis. This makes it necessary to store the sample in optimized conditions so quality is not compromised. Collection devices must be calibrated for reliable results. Various collection tools incorporating preservative reagents are currently accessible, with cell-free DNA BCT tubes (manufactured by Streck, located in La Vista, Nebraska, USA) proving effective in safeguarding against genomic DNA contamination when storing samples at room temperature (RT) for a duration of up to 14 days.[38] The K2EDTA-containing tubes, typically used, show massive release of DNA under the same conditions.

Multiple studies have demonstrated that the quality of the collected ctDNA/ cfDNA is impacted by the time-lapse between sample collection and sample processing. Szadkowska P. et al. confirmed that processing blood within 24 h after collection significantly increased the yield of isolated cfDNA.[39] Similarly, Palande V. et al. showed improved ctDNA detection rates upon instantaneous plasma separation (within two hours after blood collection) and freezing (at –80 ◦C) prior to ccfDNA isolation.[40]

Sample pre-processing requires protocols to extract or isolate the desired component. Maas SL. et al. showed that after administration of 5-ALA, PpIX accumulates in glioma-derived EVs both in the media of cell cultures and in the plasma of GB patients. Using high-resolution flow cytometry, they could detect and isolate PpIX-positive, GB-derived EVs and then further analyse their content.[41] All pre-analytical variables need to be standardized so that consistent results can be obtained and made cost-effective so they can be applied in a resource-limited setting.

Analytical processing phase and methods

Various methodologies are implied to analyse the sample to produce high-quality data. Different laboratories use different means and thus may contradict the clinical data interpretations. Because the cost of technologies for LB processing is high, LMICs like Pakistan, while trying to optimize the cost of sample analysis, may use sub-optimal technologies that produce false results, thus putting

patients at risk of an incorrect management plan for a brain tumour.

Next generation sequencing (NGS)

Next-generation sequencing (NGS) technologies provide high-throughput screening of biopsy samples and improve accuracy for mutation allele frequency (MAF), gene fusions, or DNA amplification. These methods can be applied to targeted panel systems to detect targeted ctDNA mutations and indels. However, complete tumour heterogeneity cannot be detected by the targeted panel and require whole-genome or whole-exome sequencing, which are both pricey and less sensitive. They also require extensive sample input, which may not be possible in the case of CSF.

Digital polymerase chain reaction (dPCR) and digital droplet PCR (ddPCR)

Digital PCR (dPCR) and droplet digital PCR (ddPCR) represent highly sensitive and cost-effective PCR-based techniques relevant to sequencing analysis. These methods excel in identifying allele variants or targets present in samples with minimal abundance, surpassing the limitations of conventional quantitative PCR (qPCR) approaches. Li et al. introduced an optimized ddPCR workflow, validated with tissue, to detect and measure H3.3K27M-mutant circulating tumour DNA (ctDNA) in clinically accessible cerebrospinal fluid (CSF) and plasma samples obtained from patients with diffuse midline gliomas (DMG). Addressing the challenge posed by low levels of ctDNA commonly found in brain tumours, they employed vacuum concentration of pre-amplified ctDNA, enhancing test sensitivity without compromising specificity.[42]

Nano String analysis is a novel molecular assay technique with certain benefits over PCR-based analysis as it does not require amplification with minimal risk of contamination.[43] Up to 800 molecular probes can be run simultaneously in single reactions however, it still requires normalisation of expression levels via a reference gene. FFPE can be readily used with Nano String analysis to obtain mRNA levels comparable with fresh frozen tissue samples, superior to the yield from RT-PCR. Recent experiments in its utility with liquid biopsy in lung cancers showed lower requirement for DNA compared to NGS for routine mutation testing, with strong correlations with evolution of disease.[44]

Post-processing phase

The post-processing phase is equally important. People with expertise in molecular and computational biology, genetics, and specialised clinicians are required to optimize the molecular data analyses, thus increasing the reproducibility of the results. However, their cost adds to the total cost, making liquid biopsy challenging.

Overall cost

Cost of analysis and identification of brain tumour-related serum markers is significantly hampered by lack of specificity with most markers. Various other inflammatory, autoimmune, and other cancer conditions can cause aberrations in baseline levels of these metabolites, RNA, and DNA, making specific identification of brain tumour sub-types difficult. High throughput sequencing is needed and ultimately inflates the cost of analysis per sample. A routine, affordable liquid biopsy can be achieved once specific panels of markers can be designed and implemented, allowing standardized materials to be reproduced in clinical settings.[45-47]

Conclusion

Liquid biopsy can potentially push the frontiers of personalised medicine for brain tumours. The shift towards achieving practical approaches for molecular analysis in brain tumours is essential, particularly given the significant neurological morbidity and financial burden associated with the current management. The possibility of minimally invasive cancer detection tools in brain tumour conditions could potentially revolutionise detection and follow-up rates within LMICs; a system of collection points in remote and rural areas could facilitate serum collection and use of liquid biopsy tools to help monitor and detect tumour response to treatment. Most of the developing world's population does not have quick access to MRI and neuro-radiologist facilities, nor afford the required repeated scans, resulting in missed follow-ups and recurrences. A practical liquid biopsy tool tackles both unaffordability and inaccessibility within these parts of the world, connecting these patients to more holistic neuro-oncological care.

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