Minimum Technical Preanalytical, Patient, and Clinical Context Data Elements for Cerebrospinal Fluid Liquid Biopsy: Recommendations for Public Database Submissions

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ABSTRACT

PURPOSE Blood-based liquid biopsy has enabled minimally invasive molecular profiling in patients with solid tumors. For cancers of the CNS, however, the use of peripheral blood for cell-free DNA (cfDNA) detection and sequencing has proved challenging because of scant levels of tumor-derived material shed into systemic circulation. An alternative is to use cerebrospinal fluid (CSF), which contains a greater concentration of tumor-derived cfDNA in patients with brain tumors (BTs). CSF liquid biopsy is a relatively nascent field, with critical unanswered questions regarding preanalytical variables that may affect assay performance. In an effort to identify and standardize key preanalytical variables, the Blood Profiling Atlas in Cancer (BLOODPAC) Consortium launched a BT Working Group in 2022.

METHODS We reviewed protocols for CSF sample collection and processing used by expert Working Group members at their respective academic institutions and diagnostic companies, as well as the available literature on CSF liquid biopsy. Through a collaborative and iterative process, we developed a list of key preanalytical variables for cfDNA-based CSF liquid biopsy in patients with primary and metastatic brain malignancies.

RESULTS The Working Group agreed on a recommended list of preanalytical minimum technical data elements, clinical context data elements, and patient context data elements for cfDNA-based CSF liquid biopsy in patients with CNS malignancies. A subset of variables were considered to be of critical priority and are designated as required annotations to submissions of cfDNA-based CSF liquid biopsy sample data into the BLOODPAC Data Commons.

CONCLUSION We propose a list of preanalytical variables relevant for cfDNA-based CSF liquid biopsy, with the overarching goal of encouraging routine collection and reporting of these variables in future studies.

ACCOMPANYING CONTENT

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BACKGROUND

Liquid biopsy is a test that detects tumor cells or molecules released by tumor cells in a body fluid sample.[1] Plasma cell-free DNA (cfDNA), obtained from the peripheral blood, is the predominant liquid biopsy analyte in clinical oncology.[2,3] However, in patients with primary and metastatic brain tumors (BTs), the sensitivity of blood-based liquid biopsy for detection, molecular characterization, and monitoring remains inadequate to reliably inform clinical decision making, in large part due to the blood-brain barrier.[4-6]

Relative to blood, cerebrospinal fluid (CSF) typically contains a greater concentration of tumor-derived cfDNA in patients with CNS malignancies.[7,8] Moreover, CSF paucicellularity results in minimal nontumor cfDNA derived from normal turnover of leukocytes, minimizing dilution of the cfDNA signal.[9] Key studies of CSF-based liquid biopsy over the past 5 years suggest multiple clinical applications for cfDNA analysis, such as detection of tumor somatic variants,[7,8,10-12] longitudinal disease monitoring,[13,14] detection of molecular residual disease,[15] and confirmation of initial BT diagnosis and subtype.[16] These data have bolstered research efforts to establish clinical utility of CSF

liquid biopsy in neuro-oncology, including increased industry efforts and implementation of clinical tests in a Clinical Laboratory Improvement Amendments–certified laboratory setting at select institutions. However, cfDNA-based liquid biopsy in the CSF is a nascent field, with unanswered questions regarding preanalytical variables, including technical, clinical, and patient context variables that may affect assay performance. This study describes the steps taken by the Blood Profiling Atlas in Cancer (BLOODPAC) BT Working Group to develop a list of preanalytical variables relevant to cfDNA-based CSF liquid biopsy for CNS malignancies.

BLOODPAC BT WORKING GROUP

The BLOODPAC consortium was launched in 2016 to accelerate the development, validation, and accessibility of liquid biopsy assays to better inform medical decisions and improve patient care and outcomes.[17-19] In 2017, BLOODPAC published 11 minimum technical data elements (MTDEs) for preanalytical parameters for blood-based liquid biopsy.[19]

BLOODPAC convened a BT Working Group, comprising members from academia, industry, and nonprofit foundations, in 2022 on recognition of the rapid pace at which CSF liquid biopsy is advancing, the absence of guidance regarding factors influencing assay performance, and the need for multicenter and academia-industry collaboration in this field. The Working Group aimed to identify, define, standardize, and prioritize a list of preanalytical variables relevant for cfDNA-based CSF liquid biopsy, with the overarching goal of encouraging routine collection and reporting of these variables in future studies.

The BT Working Group began by reviewing protocols for CSF sample collection and processing used by its members at their respective institutions. A total of six protocols representative of submissions by academic institutions and diagnostic companies spanning multiple assay platforms were reviewed. Through a collaborative and iterative process, the Working Group developed a list of key preanalytical variables for cfDNA-based CSF liquid biopsy in patients with primary and metastatic brain malignancies. Additionally, the Working Group assigned members to review specific aspects of cfDNA-based CSF liquid biopsy literature corresponding with the preanalytical variables. Key papers reviewed are listed in Table 1.

The Working Group synthesized this literature review and its list of preanalytical variables into recommendations outlined in this study, which were submitted to the US Food and Drug Administration (FDA) in late 2023 for review and feedback through the agency's presubmission program. The FDA's comments were integrated into the document and further reviewed by the College of American Pathologists' and the Association for Molecular Pathology. A subset of the variables were considered to be of critical priority and are designated as required annotations to any submission of cfDNA-based CSF liquid biopsy sample data into the BLOODPAC Data Commons. While at present there is little published evidence to support prioritization of certain variables over others as required, the Working Group incorporated available literature, members' experiences in their own institutions and companies, and practical concerns about feasibility of data collection when making the designation of required for each of the data elements. Submitted data must be individual patient-level, not an aggregate report of cohort-level data. Of note, liquid biopsy analytes beyond cfDNA (eg, RNA, proteins) were not the focus of this initial effort because they were considered to be further from routine clinical use in patients with BTs relative to cfDNA. Future efforts of the Working Group will incorporate these and other CSF biomarkers, as well as blood-based analytes. Importantly, the final list of variables is based not only on perceived scientific and clinical significance but also on practicality and feasibility of collection in the setting of future multicenter clinical validation studies.

RECOMMENDED PREANALYTICAL VARIABLES FOR cfDNA-BASED CSF LIQUID BIOPSY

In assay development, the preanalytical phase includes all steps that occur before the assay is run on a sample. Preanalytical variables that may influence biomarker performance include those related to the collection/processing phase (ie, technical preanalytical variables), which can be more easily controlled versus those related to the patient phase (ie, clinical context and patient context variables), which may be less modifiable. Technical preanalytical variables (Table 2; Fig 1), clinical context variables (Table 3), and patient context variables (Table 4) relevant for CSF liquid biopsy will be discussed in the following sections.

Preanalytical MTDEs

Most of the MTDEs previously published by BLOODPAC for blood-based liquid biopsy[18] were maintained. Addition of new MTDEs exclusive to CSF were decided upon by the Working Group on the basis of key differences between (1) specimen collection methods for CSF versus blood, (2) clinical and tumor characteristics of neuro-oncology patients versus other cancer populations, (3) anatomic and physiologic relationships between BTs and the CSF compartment versus those between extracranial cancers and the peripheral blood compartment, and (4) the unique turnover and cellular composition of CSF compared to blood, characterized by lower nontumor genomic DNA (gDNA) levels and rapid CSF pool turnover (approximately 150 mL four times daily).[32]

Under normal circumstances, CSF has the advantage of being paucicellular (0-5 cells/μL) containing fewer non-neoplastic cells than other fluids, such as blood (3,500-10,500 white cells/μL).[24] This advantage translates to simplified

TABLE 1. Key Studies of cfDNA-Based Liquid Biopsy in CSF Samples

Author, Year	Patient Population(s)	No. of Patients With CSF Liquid Biopsy	Assay Used
Wang et al,[7] 2015	Primary CNS malignancies (glioma, medulloblastoma, ependymoma)	N = 35	Targeted NGS panel followed by whole-exome sequencing
Li et al,[20] 2018	Non–small cell lung cancer with leptomeningeal disease	N = 45	Targeted NGS panel
Panditharatna et al,[21] 2018	DMG	N = 48	Droplet digital PCR
Mouliere et al,[22] 2018	Glioma	N = 13	Shallow whole-genome sequencing
Juratli et al,[23] 2018	Glioblastoma	N = 38	Droplet digital PCR
Pan et al,[24] 2015	Brainstem glioma	n = 7	Targeted NGS panel
Zheng et al,[25] 2019	Non–small cell lung cancer with leptomeningeal disease	n = 11	Targeted NGS panel
Miller et al,[8] 2019	Glioma	N = 85	Targeted NGS panel
Angus et al,[26] 2021	Breast cancer with leptomeningeal disease	N = 121	Targeted NGS panel followed by modified fast aneuploidy screening test sequencing
Bale et al,[27] 2021	Brain metastases (lung, breast, sarcoma, melanoma, squamous cell carcinoma of unknown origin, pancreatic), glioma, atypical teratoid rhabdoid tumor, pineoblastoma, choroid plexus papilloma, medulloblastoma	N = 137	Targeted NGS
Liu et al,[15] 2021	Medulloblastoma	n = 123	Low coverage whole-genome sequencing
Pagès et al,[28] 2022	Pediatric BTs, including medulloblastoma, atypical teratoid rhabdoid tumor, glioma, ependymoma, craniopharyngioma, germinal tumor, DMG, meningioma, nerve sheath tumor, choroid plexus tumor, pineal tumor	N = 54	Ultra low-pass whole-genome sequencing and hybrid capture panel
Cantor et al,[13] 2022	DMG	N = 24	Droplet digital PCR
Miller et al,[12] 2022	Pediatric, adolescent and young adult primary BTs, including glioma, medulloblastoma, pineoblastoma, retinoblastoma, ependymoma	N = 45	Targeted NGS
Otsuji et al,[11] 2022	Glioma	N = 25	Multiplex ligation-dependent probe amplification
Zuccato et al,[16] 2023	Brain metastases, glioblastoma, and CNS lymphoma	N = 57	Methylation profiling
Orzan et al,[10] 2023	Glioma	N = 84	Targeted NGS and droplet digital PCR
Kojic et al,[14] 2023	Pediatric BTs, including ependymoma, embryonal tumor with multilayered rosettes, neuroblastoma, and medulloblastoma	N = 12	Droplet digital PCR
Douville et al,[29] 2023	Glioblastoma, DMG, brain metastases, medulloblastoma, lymphoma, and control patients without cancer	N = 280	Repetitive element aneuploidy sequencing in CSF (real-CSF)
O'Halloran et al,[30] 2023	Pediatric BTs, including glioma, ependymoma, medulloblastoma, craniopharyngioma, choroid plexus carcinoma, atypical teratoid rhabdoid tumor, germ cell tumor, neurofibroma, hemangioblastoma, and neuroblastoma	N = 55	Low-pass whole-genome sequencing and targeted NGS
Iser et al,[31] 2024	Glioma	N = 85	Targeted NGS

Abbreviations: BT, brain tumor; cfDNA, cell-free DNA; CSF, cerebrospinal fluid; DMG, diffuse midline glioma; NGS, next-generation sequencing; PCR, polymerase chain reaction.

preprocessing and sequencing methods in comparison with other liquid biopsies. For instance, double centrifugation is not always applied in CSF protocols, and ultra-deep sequencing is not mandatory for detection of variants with targeted next-generation sequencing (NGS) approaches, unlike in plasma. However, without rigorous studies investigating preanalytic factors that influence successful profiling in CSF, our understanding is limited to those affecting cfDNA testing from other biofluids, such as plasma. The following sections describe the current state of the field regarding key technical preanalytical variables for CSF liquid biopsy and provide associated recommendations for their collection.

CSF Collection Procedure

The effect of CSF circulation is relevant when considering liquid biopsy. CSF is an ultrafiltrated fluid that flows through an interconnected network of basilar cisterns, ventricles, and extra-axial spaces, which are semicompartmentalized by brain parenchyma and meningeal membranes. The method of CSF collection must be carefully chosen to optimize the quality and quantity of the analytes of interest. Different collection methods access distinct compartments of CSF or capture CSF that has circulated to different extents. Options include the following:

TABLE 2. CSF Liquid Biopsy Preanalytical MTDEs

Number	Data Element	BLOODPAC Term	Type	Description	Required
1	CSF collection procedure	csf_procedure	Lumbar puncture Intraoperative (subarachnoid/cisternal draw, direct ventricular access, or direct from prior resection cavity) Ommaya/external catheter	Method and anatomical location used for CSF acquisition	X
2	Type of intraoperative CSF collection	intraoperative_type	Subarachnoid/cisternal Ventricular Direct from prior resection cavity	If the CSF collection was done using an intraoperative method, specify the type of intraoperative method used Note this is only required if an intraoperative method was used to collect the CSF	Conditional
3	Timing of intraoperative CSF collection	intraoperative_timing	CSF collected prior to beginning tumor dissection CSF collected after beginning tumor dissection	Indicates whether CSF was collected before or after beginning tumor dissection	X
4	CSF volume obtained at collection	csf_volume	Float (mL)	Volume of CSF fluid collected (mL) that will go to lab	X
5	Initial collection tube type	collection_tube_type	EDTA CellSave Streck ACD Other tube type Not applicable	The kind of receptacle used to collect the sample(s) taken from a patient for testing, diagnostic, propagation, treatment, or research purposes	X
6	Was there a tube transfer?	tube_transfer_indicator	Yes No	Indicates whether there was a tube transfer for the specimen	X
7	Time to tube transfer	tube_transfer_time	Float (hours)	The upper/lower limit on the amount of time, in hours, between initial specimen collection and transfer to new tube (applicable if specimen transferred from collection tube to a second, eg, preservative, tube) Note this is only required if the specimen was transferred to a new tube	Conditional
8	Temperature between tube transfers	tube_transfer_temperature	Float (°C)	Temperature, in centigrade, the specimen is kept at while awaiting transfer to new tube. (applicable if specimen transferred from collection tube to a second, eg, preservative, tube) Note this is only required if the specimen was transferred to a new tube	Conditional
9	Shipping/storage tube type	storage_tube_type	String	The kind of receptacle used for shipping/storage. Applicable if specimen transferred from collection tube to a second, for example, preservative, tube	X
10	Shipping temperature	shipping_temperature	Float (°C)	The temperature, in centigrade, at which the biospecimen was kept while it was being transported from the procurement site to its processing destination	X
11	Was this specimen centrifugated?	centrifugated_indicator	Yes No	Indicates whether the specimen was centrifugated	X
12	Centrifugation method	centrifugation_method	String	The name or description of the method used to obtain the CSF fraction sample. (eg, Novartis Protocol No. 001, 2,000 g centrifuge at 4°C with gentle deceleration). Detailed protocol should be provided as a Data Supplement when possible	Conditional
13	Time to centrifugation	hours_to_centrifugation	Float (hours)	The number of hours between the sample collection and the centrifugation into its components Note this is only required if centrifugation was performed	Conditional

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TABLE 2. CSF Liquid Biopsy Preanalytical MTDEs (continued)

Number	Data Element	BLOODPAC Term	Type	Description	Required
14	Time to centrifugation—lower limit and upper limits	hours_to_centrifugation_limits	String	The lower and upper limit on the amount of time, in hours, between the sample collection and the centrifugation into its components Note this is only required if centrifugation was performed and if the exact time is unknown	Conditional
15	Analyte isolation method	analyte_isolation_method	String	The name or general description of the method used to isolate the analyte (eg, cfDNA extraction method). Alternatively, if you have provided a protocol, put the file_name here	X
16	Time from centrifugation to freezer	hours_to_freezer	Float (hours)	The number of hours between the sample being centrifugated and the aliquot being frozen or otherwise preserved Note this is only required if centrifugation was performed	Conditional
17	Time from centrifugation to freezer—lower and upper limits	hours_to_freezer_limits	String	The upper/lower limit on the amount of time, in hours, that it took between the sample being centrifugated and the aliquot being frozen or otherwise preserved Note this is only required if centrifugation was performed and if the exact time is unknown	Conditional
18	Freezer storage temperature	storage_temperature	Float (°C)	The temperature, in centigrade, at which the aliquot was preserved and/or stored in a freezer Note this is only required if the aliquot was frozen	Conditional
19	Molecular concentration of analyte	molecular_concentration	Float (ng/mL)	If the analyte is a molecule (eg, DNA or RNA), report the observed concentration in nanograms per microliter (for molecular concentration). If the measurement is a cell count, then this is reported as cells per microliter	X
20	Absolute input amount of analyte	analyte_absolute_input	Float (ng)	Nanograms of nucleic acid (input to assay)	X
21	Assay method	assay_method	String	General name or description of the method used to characterize the analyte	X
22	Time to assay	days_to_assay	Integer	The amount of time, in days, between the date used for index and the assay used to address this analyte	X

Abbreviations: ACD, acid citrate dextrose; BLOODPAC, Blood Profiling Atlas in Cancer; cfDNA, cell-free DNA; CSF, cerebrospinal fluid; EDTA, ethylenediaminetetraacetic acid; MTDEs, minimum technical data elements.

1. Lumbar puncture-CSF (LP-CSF), obtained at the lumbar cistern, may collect CSF that has circulated throughout the spine and brain, integrating biomarkers from multiple CNS sources.
2. An indwelling CSF access device such as an Ommaya reservoir or lumbar drain allows for repeated sampling, which could provide insights into temporal CSF composition changes and the impact of circulation on biomarker levels.
3. Intraoperative acquisition during a craniotomy, including subarachnoid cisternal sampling, direct ventricular sampling, or sampling from prior resection cavity, might yield CSF from specific brain regions before it has fully circulated, possibly capturing a more localized snapshot of biomarker distribution.

Given that the circulation of CSF has the potential to significantly influence the profile of liquid biopsy analytes, it should be taken into account when selecting the collection method to ensure the most representative sampling for the intended use. Although the data comparing liquid biopsy results across different sampling methods is limited, a recent study[10] compared patients with glioma who underwent intraoperative CSF collection from a location in proximity of the tumor (n = 43, subarachnoid space; n = 2, direct from ventricle) or by LP-CSF (n = 38). Both cohorts provided matched tissue concomitant with CSF sampling, and DNA samples from CSF and matched tumors were compared by comprehensive (NGS) or targeted (droplet digital polymerase chain reaction [ddPCR]) sequencing. Consistent with smaller previous studies,[7] peritumoral CSF samples had

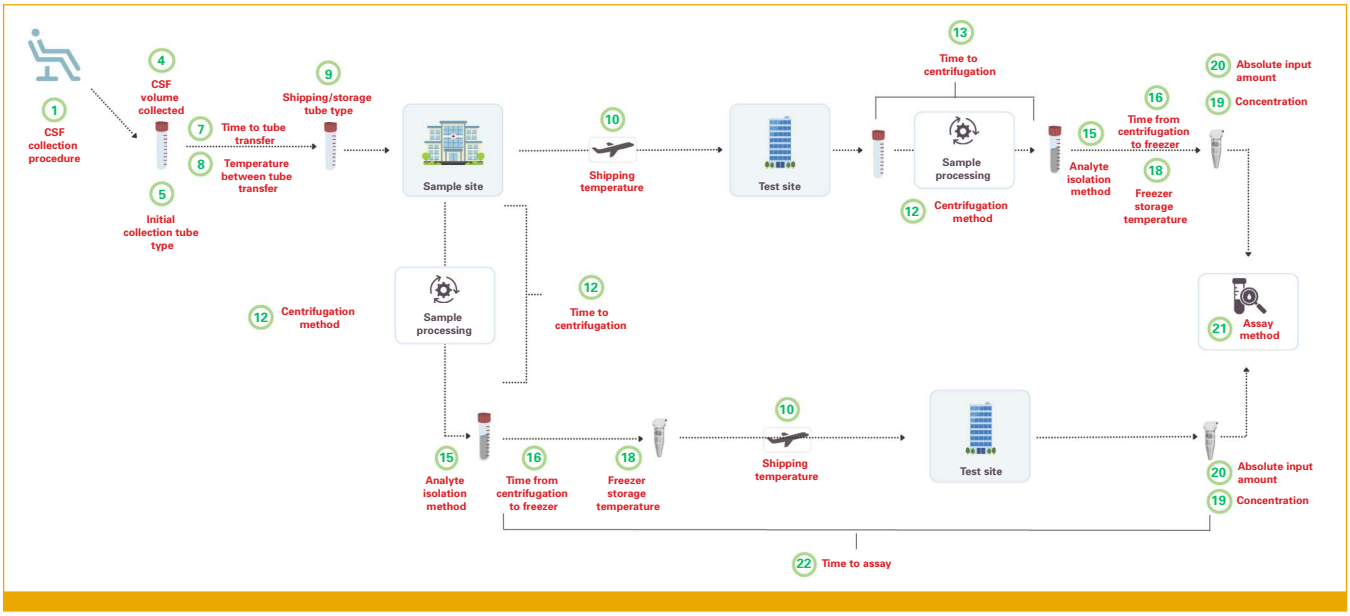

FIG 1. CSF liquid biopsy preanalytical MTDEs, see Table 2 for reference. CSF, cerebrospinal fluid; MTDEs, minimal technical data elements.

higher cfDNA concentrations and rate of mutation detection versus LP-CSF. Although additional studies are needed, we recommend that the approach taken for CSF collection and exact location of the CSF draw relative to the tumor be routinely recorded and reported.

CSF Volume

The CSF volume needed for detection of liquid biopsy analytes varies by analyte of interest, testing methodology, and the amount that is feasible for collection by patient group. With regard to cfDNA, there are no clearly established minimum volumes of CSF needed for variant detection, varying from as little as 0.4 mL up to >10 mL.[12,15,33] This wide range likely reflects the patient populations involved (eg, lower volumes collected in pediatric patients) and the method and ease of collection. Various studies have shown that successful cfDNA detection can be achieved with volumes approximating those required for standard cytology (2-3 mL).[8,9,34-36] It is likely that even lower volumes may result in useful amounts of tumor-derived cfDNA in certain scenarios, for example, with tumor-CSF contact or more active/proliferative disease. Routine, accurate documentation of CSF volume used is important to better understand how this variable affects detection performance of cfDNA and other analytes.

Collection Tube Type(s) and Associated Timings

For plasma, rigorous attention to preanalytical variables can limit non-neoplastic cell lysis, particularly leukocytes.[37-39] Cell stabilizing tubes contain preservatives that limit cell lysis and reduce release of nontumor gDNA.[40] These preservative tubes are frequently used for blood collection and shipment. Although some studies recommend their use,[41] their suitability for CSF is still being investigated. An advantage of preservative tubes is ease of transportation from collection sites to a central laboratory, which broadens the catchment area for cfDNA testing. The Streck cfDNA blood collection tube is a commonly used preservative tube, which stabilizes cell membranes for up to 14 days between 6°C and 37°C. Published studies have shown efficacy with these tubes if processed within 24 hours of collection, although detection of variants by targeted sequencing has been seen with samples up to 14 days using Streck tubes.[12,27,28] Numerous studies have also demonstrated the ability to detect tumor-derived cfDNA in CSF using sterile collection tubes that lack stabilizing preservatives. As an alternative to Streck, ethylenediaminetetraacetic acid (EDTA) tubes have the advantage of being lower-cost and readily available. However, for sterile and EDTA tubes, processing must begin more quickly. Processing typically begins immediately or within 4 hours of collection.[10,13,27] Owing to these issues, it is conceivable that some sites might collect CSF directly into the final tube or container that will be shipped or stored, whereas other sites might collect into an initial tube or container and subsequently transfer the specimen into a separate tube for shipping and/or storage (eg, a preservative tube). Therefore, the type(s) of collection tubes/container and storage/shipping tubes, as well as the time delay from initial collection to tube transfer (if applicable) need to be recorded. In general, on-site processing is preferable over shipping to a central site to mitigate risk of sample degradation.

Sample Temperature and Storage

When using nonpreservative collection, CSF should be immediately stored at approximately 4°C on ice to prevent degradation from activated blood enzymes. Samples should

TABLE 3. Proposed CSF Liquid Biopsy Clinical Context Data Elements

Number	Data Element	BLOODPAC Term	Type	Description	Required Note That All Elements That Are Not Required Are Recommended
Administrative					
i	Subject ID	SUBJECTID	String	A unique identifier for the study participant. This identifier is required, but format is not restricted in any way	X
ii	Index event	INDEX	String	Describes the index event, which is used as a temporal anchor for all temporal data within the dataset *Note that the recommendation is to use the first sample collection as the index event. However, if this date is not used, the following question (iii) should be completed to ensure all data can be included in comparative analysis where temporal data are being used	X
iii	Duration from first sample collection	SAMPCOLL	Integer	The duration (in days) from the sample collection date to the date of the index event *This field is only required if the index event is NOT the sample collection. If a different event is represented as the index event, the duration from that date to the sample collection date is required	Conditional
Diagnostic details					
1	Cancer type/diagnosis	CANTYPE	String	Include specific histopathology information	X
2	Cancer grade	GRADE	Grade 1 Grade 2 Grade 3 Grade 4	Associated cancer grade (eg, 1, 2, 3, 4)	
3	Duration from initial diagnosis	DXDUR	Integer	The duration (in days) from the initial diagnosis to the date of the index event	
Disease status and response					
4	Duration from death or last follow-up if still alive	DFUDUR	Integer	The duration (in days) from the date of death or the last follow-up to the date of the index event	
5	Disease milestone	DISMILST	Newly diagnosed/untreated Previously treated/stable Previously treated/progressing Unknown	The disease milestone the patient reached upon completion of the data request	X
6	Leptomeningeal disease (radiographically and/or cytologically proven) at the time of CSF collection?	LEPTODX	Boolean	Indicates whether leptomeningeal disease was proven with radiographical or cytological evidence at the time of the CSF collection	X
7	If cytology was performed to determine the presence of leptomeningeal disease, what were the results?	CYTORES	Negative Positive Not applicable	The result of cytology testing for leptomeningeal disease	
8	If an MRI was performed to determine the presence of leptomeningeal disease, what were the results?	MRIRES	Negative Positive Not applicable	The result of MRI testing for leptomeningeal disease	

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TABLE 3. Proposed CSF Liquid Biopsy Clinical Context Data Elements (continued)

Number	Data Element	BLOODPAC Term	Type	Description	Required Note That All Elements That Are Not Required Are Recommended
9	If an MRI resulted in a positive finding for leptomeningeal disease, what sites were involved?	MRISITE	Brain Spine Both Not applicable	The site of disease involvement, if MRI testing for leptomeningeal disease was positive	
10	Molecular alterations in tumor tissue results Yes/No	MOLALT	Boolean	Indication of whether a matched tumor tissue has been sequenced	X
11	Matched normal specimen results	MOLNORRES	Boolean	Indication of whether the matched normal specimen (eg, PBMC sample) has been sequenced	X
12	RBC count	RBCCOUNT	Number	If available, obtain results from clinical CSF studies, including Cell counts (cells/mm^3)	
13	Nucleated cell count	NUCCOUNT	Number	If available, obtain results from clinical CSF studies, including Cell counts (cells/mm^3)	
Imaging					
14	Duration from brain MRI obtained most recently (prior to CSF collection)	MRIDUR	Integer	The duration (in days) from the most recent brain MRI (prior to CSF collection) to the date of the index event	
15	Tumor location (from MRI obtained most recently prior to CSF collection)	MRITULOC	Brain tumor Spinal tumor Both brain and spinal tumors present	The location of the tumor as determined by the most recent MRI results, prior to the CSF collection	
16	Was the CSF sampled from a CSF space that is directly contacted by the tumor? (from MRI obtained most recently prior to CSF collection)	CSF CONTACT	Boolean	Indicates whether the tumor is in direct contact with the CSF space that was sampled, based on the MRI obtained most recently prior to CSF collection. In order for the answer to this question to be "YES," it should be possible to draw a single straight line through CSF from the tumor to the catheter or needle that was used for collection	
17	Is there tumor in direct contact with any CSF space (even if that space is not the same space from which CSF was sampled)?; (from MRI obtained most recently prior to CSF collection)	CSFSURF	Ependymal surface Brain surface Sulcal surface Not applicable	If yes, choose the specific CSF space(s) with which the tumor is in direct contact. If no, choose not applicable *This field is only required if the answer to 16 is YES	
18	Contrast-enhancing tumor present? Yes/No	MRI with CONTRAST	Boolean	Indicates the presence of contrast enhancement on the MRI	
Treatment					
19	Was the most recent treatment received, at time of CSF collection, systemic therapy or radiation?	TRTTYPE	Systemic therapy Radiation therapy Combined systemic therapy + radiation therapy	Defines the type of treatment received most recently at the time of the CSF collection	
20	Type of systemic therapy administered (if 19 = systemic therapy)	SYSTRTTYPE	Alkylating chemotherapy Immunotherapy Bevacizumab Targeted therapy Not applicable	If the type of treatment selected for 19 was "systemic therapy," this indicates the type of systemic therapy administered	
21	Drug name (systemic therapy used in 20)	DRUGNAME	String	If the type of treatment selected for 19 was "systemic therapy," this describes the specific drug administered	

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TABLE 3. Proposed CSF Liquid Biopsy Clinical Context Data Elements (continued)

Number	Data Element	BLOODPAC Term	Type	Description	Required Note That All Elements That Are Not Required Are Recommended
22	Duration from treatment/last treatment date	RXDUR	Integer	The duration (in days) from the last treatment date (treatment listed for variable 19) to the date of the index event	X
23	Duration from last surgery	SURGDUR	String	The duration (in days) from the last surgery to the date of the index event	X
24	Type of surgical procedure most recently performed	SURGTYPE	Biopsy Resection No surgery	Was there surgery or localized intervention performed? Include: biopsy v resection	

Abbreviations: BLOODPAC, Blood Profiling Atlas in Cancer; CSF, cerebrospinal fluid; MRI, magnetic resonance imaging; PBMC, peripheral blood mononuclear cells.

be centrifuged within 1-2 hours of collection and storage on ice. Before storage, aliquoting is critical to minimize degradation from freeze/thaw cycles, with recommended volumes of 0.5 mL or 1.0 mL per vial. Accurate documentation of freeze/thaw cycles is necessary to avoid inadvertent degradation of DNA and subsequent misinterpretation. Immediate processing is ideal, and short- and long-term storage is recommended at −80°C.

Centrifugation and Analyte Isolation Method

The most widely used method for CSF processing is centrifugation to remove cells, especially in the case of blood contamination. Multiple commercially available kits have been compared for cfDNA isolation from CSF with comparable outcomes.[43] Common methods of DNA extraction use commercially available kits such as MagMax Cell-Free DNA Isolation Kit (Thermo Fisher Scientific, Waltham, MA) which bind cfDNA to silica paramagnetic beads and the QIAamp Circulating Nucleic Acid Kit (Qiagen, Hilden, Germany) which binds nucleic acids to immobilized silica in the column. It is recommended that the CSF collection method and any potential deviations from standard protocols be carefully documented to allow for accurate and comprehensive comparison across patients, institutions, studies, and collection sites.[9] Of note, traumatic CSF taps inadvertently cause contamination of CSF with blood and will increase the amount of non-neoplastic DNA within the sample. Although centrifugation will partially alleviate this contamination, the presence of blood or blood-tinged CSF should be noted.

Molecular Concentration and Input Amount of Analyte

It is critical that the absolute quantity of input material and methodology used to quantify the analyte concentration is reported in CSF liquid biopsy research. Different platforms are available for the absolute quantification of isolated cfDNA from CSF and include Bioanalyzer Agilent technologies, Qubit, quantitative PCR, Nanodrop, and TapeStation. However, these only provide absolute quantification of the total analyte with no distinction of target analyte from background. This poses an analytical challenge because the starting input of the analyte affects downstream detection technologies. Highly sensitive RNA/DNA-based PCR assays require picograms or nanograms of total input material, whereas sequencing protocols and methylation mapping often require 5-10-fold or higher inputs. This variation may affect assay performance, interpretation of results, biomarker discovery efforts, and biological interpretation. Novel strategies and protocols for measurement of target analyte concentration and background before downstream analysis are needed to maximize reproducibility and quantify technical and biological variations in analyte concentration. For now, we recommend using normalization on the basis of the amount of DNA input (ng). This approach will establish a framework for (1) normalized comparison of results across different studies, (2) standard reporting, and (3) better understanding of starting input for different downstream applications (eg, sequencing, PCR). Accurate normalization in single and multiplexed assays will also enable better disease staging, longitudinal monitoring, and accurate and standardized reporting across institutions.

Assay Method

CSF liquid biopsy assay information should include a general name for the assay, description of the method/workflow, and a description of the analytical approach. Most commonly, these approaches have been aimed at sequence variation (eg, targeted sequencing, whole-exome/genome sequencing, or digital PCR). Method descriptions should include assay details, such as sequencing panel/loci interrogated and depth of sequencing. Furthermore, analytical approach information should include whether sequencing is tumor-informed or tumor-agnostic, as well as details for calling positives, negatives, and/or quantitation (eg, limits of detection). If comparison is made between CSF and tissue sequencing, it should be indicated whether similar platforms were used for

TABLE 4. Key Patient-Context Variables

No.	Data Element	BLOODPAC Term	Type	Description	Required Note That All Elements That Are Not Required Are Recommended
Demographics					
1	Year of birth	BRTHYR	Integer	The participant's year of birth (with an upper limit of 89 years ago)	X
2	Race	RACE	Array American Indian or Alaska native Asian Black or African American Native Hawaiian or other Pacific Islander White Other	The self-reported race of the participant	X
3	Ethnicity	ETHNICITY	String	The self-reported ethnicity of the participant	X
4	Sex assigned at birth	SEX	Female Male Intersex	The sex of the participant assigned at birth	X
5	Self-identified gender	GENDER	Female Female-to-male transsexual Intersex Male Male-to-female transsexual Other	The sex or gender assignment preferred by the participant	
Biospecimen collection					
6	Fasting status	FASTSTAT	Integer	The number of hours from the last time the participant had a meal	
Medical history					
7	Patient BMI	BMI	Number	The participant's BMI	X
8	Tobacco smoking status	SMOKSTAT	Current reformed smoker, duration not specified Current reformed smoker for ≤15 years Current reformed smoker for >15 years Current smoker Lifelong nonsmoker Smoker at diagnosis Smoking history not documented	Captures the participants's tobacco use	X
9	Tobacco smoker pack years	SMOKPKYR	Integer	Quantifies the participant's smoking status using pack years	
10	Medical history term[a]	MEDHX	String	Comorbidities for which the participant has been clinically diagnosed	

Abbreviation: BLOODPAC, Blood Profiling Atlas in Cancer.

[a]If the comorbidity data provided for a single study participant include more than one medical condition or if additional data elements related to the comorbidity are provided (eg, duration of the medical condition), an additional table will be required to accurately capture this information. For more details, see the multiple data instances examples found in the Data Supplement.

the two compartments and whether there are differences in the genes covered by the platforms' panels. Other DNA-based analyses such as methylation or copy number variation would require additional information such as conversion method for methylation or calling algorithm for copy number. More targeted approaches such as digital PCR should include locus and assay details as well as thermal cycling conditions to facilitate reproduction by other laboratories.

Control Materials

Access to sufficient quantities of clinical samples, and relevant positive and negative controls, is typically deemed

necessary for analytical validation. In the case of liquid biopsy, this may be in the form of tumor materials, blood samples, or extracted analytes. However, for CSF, there is a very limited availability of control materials because of the low occurrence of CNS-based cancers, the relative difficulty of obtaining CSF, and the small volumes obtained. As with cfDNA/liquid biopsy tests for non-CNS tumors, there is growing interest in the use of contrived samples for initial analytical validation of such tests. These may take the form of plasmid/gDNA in solution, or they can be highly fragmented gDNA from multiple genetic sources mixed into a plasma background (either artificially derived or sourced from normal/wild-type contributors). The use of CSF mimetics is not feasible because they do not mimic it in the true sense, typically being a solution of salts (Na, K, Mg, Ca) with no protein or metabolite content. Therefore, artificial CSF is not an option for understanding analytical matrix effects compared with clinical CSF samples. Alternative approaches for contrived CSF samples may include human normal CSF spiked with suitably fragmented gDNA or animal-derived CSF spiked with fragment gDNA. As described above, access to normal human CSF into which DNA could be spiked is often limited because of challenges in sample acquisition and small volumes obtained. Plasma samples may be another option, although this would require prior analytical studies illustrating equivalence between plasma and CSF derived analytes.

Clinical Context MTDEs

In addition to preanalytical factors, collecting clinical context variables (Table 3) that may affect liquid biopsy results is crucial. The temporal relationship between initial BT diagnosis and the CSF collection (eg, number of months between these events) and the tumor's histological subtype and grade should be collected. Of note, a small minority of patients may not have a tissue-proven diagnosis (eg, brainstem glioma that is diagnosed on a radiographic basis). If feasible, survival data should be collected and correlated with liquid biopsy results.[23] When available, the results of routine clinical studies from the CSF sample should be included, such as cell counts with differential and cytology. Beyond these basic patient characteristics collected in most clinical research studies, the following sections detail additional clinical context variables relevant for CSF liquid biopsy studies.

Tumor Anatomic and Radiographic Characteristics

Anatomic and radiographic features such as location(s) within the brain, size of the tumor, extent of contrast enhancement, and presence of leptomeningeal disease (radiographically and/or by positive cytology) can affect levels of tumor derived analytes in CSF.[7,8,10,44] Thus, these parameters should be detailed to the extent possible. Additionally, direct contact of the tumor with a CSF space, such as an ependymal surface, brain surface, or sulcal surface, should be noted, including whether the specific CSF space used for sample collection is in direct contact with the tumor. Importantly, a myriad of potential radiographic features of disease could potentially correlate with the yield of a CSF liquid biopsy. Although we have recommended only those variables that we consider to be highest priority at this time and relatively easy to acquire, there are others that could be explored and have potential value (extent of edema, presence of diffusion restriction, tumor volume, etc). We encourage collection of as many radiographic variables as possible from available imaging so that future analyses may examine potential correlations. We also suggest that investigators share actual imaging files on request.

Tumor/Disease Status at the Time of CSF Collection

In plasma, cfDNA detection and quantity is used to track disease burden over time across numerous solid tumors.[45-50] In CSF, because of limited fluid accessibility, there have been few prospective or large retrospective studies with longitudinal sampling at set time points to evaluate the relationship between tumor burden and disease status. However, there are a few examples showing the correlation between disease burden and CSF cfDNA status, including using shallow whole genome sequencing of CSF cfDNA in medulloblastoma,[15] whole-exome sequencing along with customized ddPCR on CSF cfDNA in medulloblastoma,[51] ddPCR for H3K27M on CSF in diffuse midline glioma (DMG),[13] and detection of tumor-derived cfDNA by targeted NGS in patients with glioma.[8,10] Although radiographic assessment of disease status is challenging in glioma and other primary BTs, establishing and recording tumor/disease status (ie, progressive *v* stable disease) at the time of CSF collection is critical to validating the clinical utility of the assay. At minimum, we recommend noting whether, at the time of the CSF collection, the disease is (1) newly diagnosed and untreated, (2) previously treated and stable, or (3) previously treated and progressive.

Most Recent Anti-Neoplastic Therapy and Surgery Before CSF Collection

Although prospective studies looking at the timing of treatment (including surgery, radiation, and/or systemic therapy) in relation to cfDNA positivity in CSF for patients with primary BT have been limited to medulloblastoma[15] and DMG,[13] the effect of treatment on plasma cfDNA dynamics is well established in extracranial cancers. For example, cfDNA clearance after the administration of curative-intent treatment can predict prognosis and is increasingly being integrated as a biomarker in clinical trials across solid tumors.[52-59] Furthermore, effective treatment may result in significant tumor cell death leading to transiently increased release of tumor-derived cfDNA,[60] which may affect assay results. Thus, annotation of prior anti-neoplastic therapies, particularly the line of treatment most recently received before CSF collection, is important. Similar consideration should be given to the neurosurgical procedure most recently performed before CSF collection.

Availability of Molecular Profiling From Tumor Tissue

In early assay development and validation, comparing CSF cfDNA sequencing with tumor tissue sequencing from the same patient, ideally from contemporaneous samples, is crucial for interpreting mutations. This direct comparison will confirm whether CSF cfDNA can be used as a surrogate for tumor biopsy when tissue is difficult or unable to be obtained and whether CSF may detect clinically relevant mutations missed by tissue sequencing. A study by Miller et al[8] looked at the concordance between CSF and tumor pairs that were collected within 3 weeks of each other and found that these patients had close to identical mutational profiles. Nonetheless, as the interval increased between the tumor and the CSF collection, there was increasing genetic divergence. Moreover, it has not been established whether all disease-defining and oncogenic alterations commonly seen in primary BTs are observed at the same frequency in NGS of CSF cfDNA. For example, alterations in the *TERT* promoter are notoriously difficult to detect with NGS because of their guanine-cytosine rich regions that encourage secondary structure formation and prevent proper amplification.[61] Hence, to properly interpret the absence of disease-defining/oncogenic drivers in CSF when present in matched tissue that has been sequenced, it is critical to view these results in the context of the specific gene(s)/mutation(s) in question. When possible, it is helpful to have sequencing results of any matched normal blood samples because this helps filter out mutations representative of germline single nucleotide polymorphisms or clonal hematopoiesis.

Patient Context MTDEs

Patient context MTDEs consist of patient factors at the time of biospecimen acquisition that may affect assay results. The final list of patient context variables is provided in Table 4. These variables include basic demographic information.

SUMMARY

We described the BLOODPAC BT Working Group process for selecting preanalytical MTDEs for cfDNA-based CSF liquid biopsy in neuro-oncology and encouraged academia and industry to embrace these standards for robust cfDNA assay development. Although practical and broadly applicable, these lists may not include all assay-specific variables. When feasible, we recommend reporting quality control measures or standards, any specific handling procedures or precautions, information of sample quality assessment, and any deviations from these processes. Future directions for the Working Group include developing control/reference materials to facilitate multicenter studies for clinical validation of CSF liquid biopsy assays and expanding our efforts to other analytes beyond cfDNA and blood-based liquid biopsy (eg, focused ultrasound).[62]

This report is limited by the paucity of published evidence available to support the absolute and relative effect of each of the recommended preanalytical variables on downstream results of cfDNA-based CSF liquid biopsy. Our recommendations and those of other working groups should enable larger, multicenter studies to answer key questions about which preanalytical variables are most critical to obtaining clinically actionable results. Additionally, we recognize that similar efforts to ours are occurring across the field,[63,64] each one with its own niche and important contributions to the field. Our report is unique in that our list of preanalytical and patient context variables are meant to serve as the official, BLOODPAC required and recommended variables for data submitted to the BLOODPAC Data Commons. In addition, our recommendations are specific to (1) CSF (not blood or other fluid compartments) and (2) cfDNA-based assays (not other analytes).

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DISCLAIMER

The work described here was done through the BLOODPAC Consortium, which is a not-for-profit consortium consisting of members from industry, academia, not-for-profits, and US Government agencies, including companies that sell liquid biopsy assays, companies that use liquid biopsy assays as companion diagnostics, organizations that do research related to liquid biopsies, organizations that conduct clinical trials involving liquid biopsies, and agencies that develop policies and procedures related to liquid biopsies. In addition, some of the authors are employed by companies in the liquid biopsy field, have stock in companies in the liquid biopsy field, or consult with companies in the liquid biopsy field. The authors worked together collaboratively to develop consensus opinions and the authors do not have any particular or specific conflict with the work described in this paper, beyond those enumerated in the Conflict of Interest statement.

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