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Topical Review

Collection and Analyses of Cerebrospinal Fluid for Pediatric Translational Research

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ABSTRACT

Cerebrospinal fluid sample collection and analysis is imperative to better elucidate central nervous system injury and disease in children. Sample collection methods are varied and carry with them certain ethical and biologic considerations, complications, and contraindications. Establishing best practices for sample collection, processing, storage, and transport will ensure optimal sample quality. Cerebrospinal fluid samples can be affected by a number of factors including subject age, sampling method, sampling location, volume extracted, fraction, blood contamination, storage methods, and freeze-thaw cycles. Indicators of sample quality can be assessed by matrix-associated laser desorption/ionization time-of-flight mass spectrometry and include cystatin C fragments, oxidized proteins, prostaglandin D synthase, and evidence of blood contamination. Precise documentation of sample collection processes and the establishment of meticulous handling procedures are essential for the creation of clinically relevant biospecimen repositories. In this review we discuss the ethical considerations and best practices for cerebrospinal fluid collection, as well as the influence of preanalytical factors on cerebrospinal fluid analyses. Cerebrospinal fluid biomarkers in highly researched pediatric diseases or disorders are discussed.

Introduction

Translational research is a multidimensional concept that implements new research findings from bench to bedside.[1] Bio-banking of biologic samples is a fundamental component of translational research. A bio-bank is not only a tool for the storage of biologic samples for future research but also a means for collectively distributing conserved, documented, and methodically handled quality samples for translational research.[2-4] Bio-banks should contain clinically relevant data and allow researchers to access biologic samples, including cerebrospinal fluid (CSF).

Tissue sampling and bio-banking are critical for pediatric health studies,[3,5-7] and thus, we review CSF collection, processing, storage, and shipping. We also include examples of CSF analyses, including their clinical implications. Preanalytical factors (i.e., sample collection, handling, storage, and transport) and protocol variability can account for 46% to 68.2% of all laboratory errors[8] and more than 60% to 90% of diagnostic errors.[9] Specifically, the measurement of biomarkers can vary as much as 20% to 36%.[10-12] Unreliable sample analysis as a direct result of preanalytical errors and protocol variability can have a negative impact on patient care.[8] As such, it is imperative that comprehensive and universally accepted protocols be mandated.[12] We present practices for CSF handling so as to maintain high-integrity samples.

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Cerebrospinal fluid

CSF is an important diagnostic substrate that can provide health care professionals with essential information regarding the current pathology of the brain, spinal cord, and meninges, making its analysis a critical component of clinical practice.[13-18] CSF is typically a clear and colorless fluid that consists of components derived from both the blood and the central nervous system (CNS).[15,16,18-21] The volume of CSF is approximately 40 mL in the term neonate and ranges between 65 and 140 mL for children aged four to 13 years.[21] CSF is separated from the blood by two barriers, the blood-brain barrier and the blood-CSF barrier.[13,18,19,22,23] Up to 75% of CSF is formed in the choroid plexus of the ventricles.[21,24-26] Reabsorption of CSF into the venous system occurs through bulk flow by the arachnoid villi in older children and adults[15,19,21,22,25-28] and absorption through the nasal lymphatics in young children as the arachnoid absorption system becomes fully functional only after age 18 months.[24,28] Absorption also occurs at cranial and spinal nerve sheaths, at the cribriform plate, and at the adventitia of cerebral arteries and CNS capillaries.[24,29] CSF consists of proteins (approximately 20% brain derived[16,18,26,30]), amino acids, electrolytes, inorganic salts, sugars, catecholamines, steroids, antibacterial factors, metabolic by-products, and organic acids.[21,23,27,31]

Ethical considerations

In general, it is not appropriate to conduct research in children that exceeds minimal risk unless it will directly benefit the child or, if no direct benefit is expected, it will provide knowledge that is essential for understanding the condition or disease from which the child suffers.[32] Minimal risk in a vulnerable population, such as children, includes considerations of pain, distress, and psychological harm including anxiety or guilt.[32] Collection of CSF in live patients is invasive and is therefore considered above minimal risk.[32] Means of decreasing potential harms associated with CSF collection include minimizing the number of sample collections, conducting sample collection concurrently during other diagnostic or treatment procedures, coordinating research sample collections at the time of clinically mandated sample collection, and implementing methods to decrease anxiety surrounding the procedure.[32,33] Regardless of the approach, it is essential that collection, processing, and analytic methods are consistent to ensure high-quality samples that are optimized for both clinical and research purposes.

Guidelines[34-37] stipulate ethical conduct for research and include sections on vulnerable populations such as children. Ethical considerations regarding CSF collection in children are very similar to those related to the collection of other types of specimens. Please see previous reviews by our group for detailed discussion on ethical considerations for sample collection in children.[3,6,7]

CSF collection methods

CSF can be obtained by lumbar puncture (LP), via a ventriculoperitoneal shunt (VPS), via an external ventricular drain (EVD), intraoperatively, or through postmortem collection. Regardless of the means of collection, detailed documentation regarding sampling and processing must occur to ensure generalizability and reliability (Tables 1-3). Determination as to the appropriateness of a particular method requires consideration of potential complications and contraindications (Table 4).

TABLE 1.
Cerebrospinal Fluid Collection: *In Vivo* Factors

Factor	Rationale	Recommendation
Timing of CSF sample collection[17,23,38-40]	Biomarkers affected by circadian rhythm will be expressed differently throughout the day	Record time of day of sampling. Strive to collect samples at same time of day
Fasting[38-41]	No data to indicate that fasting has an effect on biomarker levels except in GLUT1 deficiency	In the case of novel biomarker studies fasting status should be recorded to evaluate any influence on biomarker levels Specifically for GLUT1 lumbar puncture should be performed after 4- 6 hours of fasting
Age[14,15,17,31,42-45]	Protein concentrations are higher in newborns and older adults, lowest in children, increasing again in adolescence and adulthood. Biomarker levels (e.g., total tau, glial fibrillary acidic protein, neurofilament light, albumin, and immunoglobulin G) correlate with age. Age-related pediatric references values for total biopterins, BH2, BH4, and NEO. There are 30 proteins with >20% change in concentration between older and younger children	Use age-matched reference populations
Patient activity level[46]	Higher CSF protein concentrations in patients when lying down compared with sitting. Influence of thoracoabdominal pressure	Record levels of patient activity prior to sample collection
Patient history[23,41]	Alcohol and cigarettes can alter concentrations of homovanillic acid and serotonin, possible markers of neurodegenerative disorders. Some medications can alter CSF proteins (e.g., drugs used for treatments, such as immunomodulatory agents and methylprednisolone, influence biomarker expression)	Take a detailed patient history including extracurricular and prescription medication usage that includes types of medication and duration of use for at least one year preceding sample collection. If CSF glucose is under investigation obtain a blood glucose value before lumbar puncture to evaluate stress-related hyperglycemia
Gender[17]	Hormonal influence on biomarkers	Gender-matched reference populations
Ethnicity[17]	Biomarkers can be influenced by genetic status (i.e., higher IgG concentrations in African Americans than Caucasians)	Reference populations for ethnical subgroups

Abbreviations:
CSF = Cerebrospinal fluid
GLUT1 = Glucose transport protein type 1

TABLE 2.
Cerebrospinal Fluid Collection: Preanalytic Factors

Factor	Rationale	Recommendation
Anatomic location of sampling [14-17,23,30,31,38,42,47-51]	Brain and blood protein concentrations in CSF vary based on location due to rostral-caudal protein concentration gradient (e.g., 2.5 times higher protein concentration in LP samples than in ventricular samples)	Comparisons should be made only between samples collected in the same manner. Reference populations must be identified with similar characteristics (i.e., reference samples for patients with TBI may include intraoperative, EVD, or VPS collection from hydrocephalic patients or patients with an unruptured SAH
Volume and fraction of CSF collected [14-18,23,25,30,38,42,47,49,50,52]	Influence of rostral-caudal concentration gradient. Collection of a proportion of volume based on age or patient size may alter biomarker data as samples collected from smaller patients are more likely to draw CSF from a more rostral location Insufficient volume of CSF leads to false-negatives	Record total volume and fraction(s) sent for bio-banking. Collect a standard sample volume. If numerous fractions collected either utilize the same fraction for biomarker analysis or combine all samples and divide into aliquots before analysis Ensure adequate volume for analysis is collected. In adults, false-negative rates decrease from 32% to 3% as sample volume increases from 2.5 cc to 10 cc
Intermittent versus continuous sampling (EVD)[48,53]	Concentration of biomarkers increased twofold and total volume of CSF drained decreased by half when CSF intermittently drained	Record the manner of drainage utilized
Number of RBCs in sample [16,17,20,23,26,38,43,47,48,51,54-57]	Indicator of sample contamination. Contaminated samples that are not centrifuged rapidly after sample collection must not be utilized	Record the number of RBCs in sample. Record time to centrifugation. Cool immediately upon collection. Utilize tools to identify presence of hemoglobin chains, an indicator of blood contamination (i.e., MALDI-TOF-MS)
Type of sampling tube [16,17,23,38,39,47,48,51,58-63]	The type of tube material can influence protein binding. Polypropylene tubes are the recommended tube for sampling and storage	Record the product manufacturer
Time between sampling and storage[14,17,20,38,48,55,56,64,65]	Time delay is highly variable due to the inconsistency in procedures at different sites. Time delays can result in deleterious effects due to sample contamination and protein degradation	Minimize time delays for transport to laboratory. Immediately store sample on ice and transport while cooled
Freeze-thaw cycles[16,17,38,48,57,60]	Freeze-thaw cycles lead to protein denaturation.	Avoid freeze-thaw cycles. Store many samples at lesser volumes. Record biomarker levels reflective of sample quality
Collection of comparative blood samples[17,39,47]	Important to compare levels of blood-derived proteins to identify blood-brain barrier dysfunction. Important for determination of intrathecal origin of biomarker	Collect matched serum or plasma at the time of CSF sampling. Collect in vacuum tubes using EDTA in dried format
Use of additives[16,17,38,48,56,57]	Additives such as protease and phosphatase inhibitors may result in artifacts during protein identification and quantification	The use of additives is not recommended unless for specific research purposes
Analysis and interpretation of data from CSF samples[66]	WBC values are often presented in terms of means, standard deviation, and range. The frequency distributions for WBC from CSF samples are sometimes markedly skewed when these are used. Owing to this, sometimes is difficult to use the information to determine the probability that a particular sample is abnormal	The cell count, and other determinations from CSF, should be evaluated within clinical context of the individual case to avoid false interpretation. Percentiles should be constructed for each group to determine how many normal patients have less than a particular number of cells in their CSF

Abbreviations:
CSF = Cerebrospinal fluid
EDTA = Ethylenediaminetetraacetic acid
EVD = External ventricular drain
LP = Lumbar puncture
MALDI-TOF-MS = Matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry
RBC = Red blood cell
TBI = Traumatic brain injury
SAH = Subarachnoid hemorrhage
VPS = Ventriculoperitoneal shunt
WBC = White blood cells

TABLE 3.
Cerebrospinal Fluid Collection: Postmortem Factors

Factor	Rationale	Recommendation
Timing of sample collection relative to postmortem interval[7,67,68]	Cell counts, protein concentration, and other components increase with increasing postmortem interval. Low postmortem interval associated with higher-quality tissue	Record time of sample collection. Collect samples within hours after death. To prevent delay obtain consent for sample procurement in advance
Body storage temperature[67]	More striking increases in cell and protein counts are discernible in bodies stored at room temperature	Record the temperature at which the body is stored and for how long
Postcollection sample temperature[7]	Maintaining the integrity of biochemical molecules	Snap freeze and store at −80°C
Agonal factors (including coma, pyrexia, hypoxia, multiple organ failure, prolonged death, seizures, dehydration, hypoglycemia, respiratory arrest)[7]	Alteration of gene expression for proteins, proteolytic activities, and ribonucleic acid quality	Record type of and duration of agonal factors

TABLE 4.
Potential Complications and Contraindications of Cerebrospinal Fluid Collection Methods

CSF Sampling Method	Potential Complications	Contraindications
LP[13,27,31,47,54,69-72]	Epidural and subdural hematoma Cortical blindness Cervical spinal cord infarction with respiratory arrest and flaccid tetraplegia Cranial nerve palsy Meningitis Post-LP headache Lower back pain Bleeding at the puncture site Cauda equina syndrome Herniation of the brain	Presence of a VPS Cardiorespiratory compromise Increased ICP Infection present in area where needle must pass through Lack of mature connective tissue in epidural space Bleeding diathesis Thrombocytopenia Anatomic spinal cord abnormalities Previous surgery at LP site
VPS[24,70,73,74]	Bleeding at puncture site CSF leakage Damage to valve Introduction of infection Overdrainage Obstruction Mechanical failure Loculation of ventricles Abdominal complications (ascites, abdominal pseudocyst, perforation) Tinnitus (at high altitudes)	Lack of sampling reservoir
EVD[75-78]	Infection Misplacement Hemorrhage Ventriculitis Malfunction Obstruction Renal failure due to severe dehydration	Coagulopathy Unrepaired ruptured cerebral aneurysm (relative contraindication)
Intraoperative[79-81]	CSF rhinorrhea CSF leak CSF accumulation in extra-axial space	

Abbreviations:
CSF = Cerebrospinal fluid
EVD = External ventricular drain
ICP = Intracranial pressure
LP = Lumbar puncture
VPS = Ventriculoperitoneal shunt

Lumbar puncture

Most often CSF is collected by LP at the L3-S1 level.[13,16,17,25,46,47,54,82] As the spinal cord terminates at the L2-L3 level in children, sampling at or below the L3 level should prevent damage to the spinal cord.[25] Use of an atraumatic needle (i.e., Sprotte or Whitacre) with the bevel placed parallel to the spine is better tolerated by patients and results in fewer postprocedural headaches.[17,47,48,69,82,83] The type of needle does not alter biomarker levels.[17] A maximum of 5 mL CSF is to be collected from pediatric patients.[55,82] The CSF should be drawn with a polypropylene syringe or alternatively allowed to flow under gravity and collected into sterile polypropylene tubes.[17,47,48,55]

An important consideration when obtaining CSF via LP is the rostrocaudal concentration gradient that exists for total protein, metabolites, and cell counts due to diffusion of plasma constituents into the CSF.[14-17,23,30,42,49-51] As a result the concentration of plasma-derived proteins may be as much as 2.5 times higher in the lumbar sac than in the ventricular fluid,[15,23] whereas the concentration of many brain-derived proteins is consistent throughout the CSF pathway or decreased in the lumbar region (i.e., tau protein).[23,30,51] As such, the CSF volume, portion of CSF, and other factors (Table 2) utilized for analysis may influence the concentration of biomarkers and diagnostic results.[14-18,23,30,42,49,50] Inaccurate results may be observed if volumes differ significantly between samples.

Ventriculoperitoneal shunt

A VPS diverts CSF when the intrinsic pathway is insufficient. CSF can be collected from the VPS reservoir by a VPS tap. This procedure has potential complications (see Table 4) and requires consultation with a neurosurgeon.[70] Research comparing CSF data from an LP versus a VPS has indicated that CSF obtained by LP is a more sensitive detector of CNS tumor cells.[84,85] Use of VPS fluid may be indicated when CSF flow is obstructed.[84]

External ventricular drain

An EVD is a catheter that is inserted into the ventricular system and allows for intracranial pressure monitoring and drainage of CSF.[75] When an EVD is in place, CSF is to be drawn from the ventriculostomy catheter.[48] There are two types of drainage methods, continuous and intermittent.[48,53] CSF that is drained continuously results in the removal of a greater volume of CSF, lower CSF traumatic brain injury biomarker concentrations, and lower mean intracranial pressure.[53] It is hypothesized that the volume drained by way of continuous drainage is greater because CSF has a

tendency to drain through the open ventriculostomy catheter as opposed to internal methods.[53] Although there is inadequate data to describe the mechanism of diluted CSF, it is likely associated with the rate of production or reabsorption of the CSF, the proteins, or a combination of the two.[53] Regardless, it is imperative that the drainage method be recorded at the time of CSF sampling to account for possible discrepancies.[48,53]

Intraoperative collection

CSF samples collected intraoperatively are commonly studied in pediatric patients with posterior fossa brain tumors. Specifically, CSF collected from the cisterna magna intraoperatively, as opposed to other collection methods, reduces variability due to the site of sampling, provides more reliable data due to the proximity of the sampling to the tumor site, and also decreases the likelihood of false-positive LP findings because of mechanical disruption of the tumor.[52] Furthermore, intraoperative CSF sampling may negate the need for additional CSF collection by LP, which is an invasive secondary procedure that may result in a number of potential complications (Table 4).[52]

Postmortem collection

Postmortem CSF is easily collected from the cisterna magna by suboccipital puncture during autopsy procedures.[86,87] Postmortem analyses are commonly undertaken for the determination of blood glucose concentration before death[88] and for research into sudden infant death syndrome.[87] It is important to refrain from comparisons between living controls and samples collected at postmortem as metabolite levels in autopsy controls have been found to be threefold higher than in living controls.[89] Postmortem samples must be collected, processed expeditiously, and have sample collection factors (Table 3) meticulously documented as the concentration of many CSF compounds are altered with an increasing postmortem interval.[67,68,90]

CSF processing

The role of preanalytic factors

Preanalytic factors (Table 2) can influence hemolysis and lead to changes in the proteome.[8,9,20,38,56,57] Changes in the proteome have been observed as early as 30 minutes in uncentrifuged CSF samples left at room temperature.[57] CSF should only be collected in polypropylene tubes, and polystyrene and glass tubes should be avoided.[16,17,23,38,39,47,48,51,55,58-61] Typically, additives, such as protease and phosphatase inhibitors, are not recommended unless specified for research purposes.[16,17,48] An additive that may be considered for cellular stability is TransFix. Originally developed to stabilize whole blood for extended processing periods, TransFix has a longer shelf life and is more readily available than other cellular stabilizers.[58] When combined with ethylenediaminetetraacetic acid (EDTA), an aminopolycarboxylic acid that chelates divalent cations, TransFix helps to stabilize CSF for greater than 18 hours and may be useful for delayed analysis and shipping.[58] The addition of dithioerythritol and diethylenetriamine pentaacetic acid, an expanded version of ethylenediaminetetraacetic acid, followed by immediate storage of samples at −70°C has been used to prevent oxidation of highly unstable metabolites in the pterin biosynthetic pathway.[42] These antioxidants help to stabilize samples for at least six months at −70°C and up to two hours at room temperature.[42]

Serum-containing medium also prevents cellular degradation in CSF for at least five hours after collection, and although its effects are not as long-lasting as those of TransFix, it may be useful for situations wherein delayed processing is not preventable.[58,64] After collection CSF samples should be immediately placed on ice and rapidly transported to the laboratory for processing.[14,17,20,38,48,56,62,65] Regardless of the type of future analysis, storage procedures for all CSF samples must be initiated expeditiously, ideally within 30 minutes and absolutely no greater than one to two hours after collection to diminish the incidence of hemolysis and protein degradation.[14,17,48,56,62,65]

CSF preparation and storage

Centrifugation variables (i.e., time, force, and temperature) will impact the degree of cellular separation in fluid samples.[4] The recommended force at which to centrifuge a CSF sample ranges from 250 × *g* to as high as 5000 × *g* for between five and 15 minutes[16,17,20,39,47,48,53,56,57,61-63,91]; however, the ideal spinning condition based on consensus recommendations is to spin at 2000 × *g* for 10 minutes, preferably cooled, or 400 to 450 × *g* when cells are to be preserved for analysis.[17,47,64] Following centrifugation the supernatant is separated into small aliquots, to prevent unnecessary freezing and thawing, and stored in chilled polypropylene tubes with a preferred minimum volume of 1 mL but no more than 75% of the maximum volume so as to prevent freeze-drying.[17,47,62]

The stability of biomarkers varies at different storage temperatures. Oligoclonal immunoglobulin G bands, an important biomarker for multiple sclerosis and other neuroinflammatory diseases, remain stable for years at −20°C. In contrast, the protein cystatin C and concentrations of excitatory amino acids are compromised at −20°C, which may be misinterpreted as part of an underlying biologic process.[36,37] Freezing slows protein degradation, indicating that CSF should be immediately frozen following centrifugation and stored between −70°C[42,55] and −80°C.[13,16,17,34,36-38,47,48,55-57,60,92-94] Snap-freezing by way of liquid nitrogen has been recommended before storage at −80°C.[42,48,57] Regardless of the freezing method chosen, the aliquoted samples must be labeled with water- and frost-resistant labels.[17] Bar-coded cryovials are excellent alternatives, albeit more expensive.[3,17]

Freeze-thaw cycles have been found to lead to protein denaturation, negatively influencing biomarker concentrations, and should be avoided when possible.[16,17,48,57,60] For example, concentrations of amyloid-β decrease following a one-time freezing and decrease a further 20% after three more thawing cycles.[60] Storing many samples of lesser volume is recommended to help reduce freeze-thaw cycles.[17,57]

Thawed CSF samples should be shaken and re-centrifuged to ensure that potential biomarkers within the sample are homogeneously distributed.[48,95] Thawing should occur slowly on wet ice; however, rapid thawing is a suitable practice as long as the temperature does not exceed 40°C and does not occur for longer than 10 minutes so as to avoid unacceptable levels of evaporation.[48,95] Measures of sample quality assurance have been developed and include matrix-assisted laser desorption/ionization-time-of-flight (MALDI-TOF) mass spectrometry and surface-enhanced laser desorption/ionization-time-of-flight mass spectrometry, which are able to detect blood contamination and are indicators of less-than-ideal storage conditions (i.e., cystatin C fragments and oxidized proteins such as human transthyretin).[38] Research with porcine CSF has demonstrated that prostaglandin D-synthase is strongly influenced by storage procedures and may also be a useful tool for determining sample quality.[57]

TABLE 5.
Normative Findings From Pediatric Cerebrospinal Fluid[21,27,43,96,97]

Age	WBC $\times 10^9$/L Mean (Range)	RBC $\times$ 10^{12}/L Mean (Range)	Glucose (mmol/L) Mean (Range)	Protein (mmol/L) Mean (Range)
Premature infants	0.0051-0.009 (0.0003-0.028)	-	2.8-3.7 (1.3-10.8)	1.0121.15 (0.27-2.6)
Term newborn	0.0082 (0-0.022)	-	2.9 (1.9-6.6)	0.9 (0.2-1.7)
0-4 weeks	0.0045-0.011 (0-0.035)	0.0000955 (0-0.0000236)	2.5-3.7 (1.7-9.9)	0.754-0.84 (0.1581.89)
4-8 weeks	0.0031-0.0071 (0-0.025)	0.0000755 (0-0.0000645)	2.6-2.7 (1.6-3.6)	0.589-0.59 (0.055-1.21)
>8 weeks	0.0023-0.003 (0-0.005)	0.0000312 (0-0.000030)	3.2-3.4 (1.9-3.9)	0.28-0.392 (0.07-0.71)

Abbreviations:
RBC = Red blood cells
WBC = White blood cells
(-), No values found.

CSF shipping

The shipping of CSF samples should be initiated early in the week to avoid any delays due to weekend transfers.[47] Transportation must occur on dry ice and with a sufficient amount of dry ice to account for unforeseen delays.[34,55] When transportation over great distance is required, samples can be lyophilized before shipment and then later reconstituted by the addition of deionized water to the original volume.[34,94]

CSF analysis

Once collected and processed a number of techniques are implemented to analyze the CSF. Protein separation occurs concurrently or before the identification and quantification of possible predictive biomarkers.[31] Fractionation may be essential for the detection of new diagnostic biomarkers. To remove the most abundant CSF proteins and allow for the detection of less abundant and low-molecular-weight proteins and peptides, one can use either molecular weight cutoff filters with a cutoff above a predetermined threshold or antibody-based depletion columns. Alternative methods of protein separation include chromatography and electrophoresis; however, these separation methods may result in sample dilution.[18] Two-dimensional gel electrophoresis was previously the gold standard; however, this method is being replaced with gel-free separation methods including liquid chromatography, high-performance liquid chromatography, and capillary electrophoresis.[31]

Particularly useful for the analysis of low-molecular-mass peptides and proteins are mass spectrometry techniques including MALDI-TOF, surface-enhanced laser desorption/ionization-time-of-flight mass spectrometry, as well as electrospray and Fourier transform ion cyclotron resonance.[31] MALDI-TOF in particular has been highlighted because of its fast and accurate processing capacity, relatively low cost, ability to analyze low-mass proteins and peptides, automation, and low sample volume (10 μL) requirement.[38] Liquid chromatography with electrochemical and fluorescence detectors is utilized for the analysis of serotonin and catecholamine metabolites.[49]

Clinical analysis

Cellular identification

Clinical analysis of CSF samples is typically utilized for diagnostic purposes, but the analyses also provide basic information on sample quality and ongoing pathologic processes, which is imperative to supplement research questions. Total red blood cell and white blood cell (WBC) counts require one drop of unspun CSF, and deviation from the normative count can indicate a number of disease states (see Tables 5 and 6).[21,25-27] A Wright-stained smear can be done to differentiate WBCs.[21] CSF pleocytosis, defined as more than 5 $\times$ 10^6 leukocytes/L, is indicative of a possible infection or inflammatory process.[64] Leukocyte degradation of 15% to 62% can occur in the first two hours after CSF collection resulting in incorrect normocellular diagnosis secondary to delayed processing.[64] To improve the reliability and validity of cellular counting flow cytometry is recommended.[64] Furthermore, flow cytometry allows for the differentiation of leukocytes by way of immunophenotyping.[64] Defining the type of cell present in CSF samples provides more information than simply identifying the number of cells.[108] For example, children with noninflammatory diseases have a greater number of T lymphocytes and very low numbers of B lymphocytes, whereas increased levels of B lymphocytes is indicative of humoral or autoantibody-mediated processes.[108]

Diagnostic testing

Glucose, lactate, and protein concentrations are also evaluated compared with normative values to determine possible infectious processes (see Tables 5 and 6).[21,25-27] Tests for the detection of bacterial and viral antigens may include latex agglutination and DNA amplification (i.e., polymerase chain reaction).[21,25,43] Gram staining is a simple, inexpensive, rapid, and accurate (sensitivity = 97.5%, specificity = 94.1%) method utilized to determine the presence of infection and to identify microorganisms.[109,110] Ziehl-Neelsen staining is utilized for the detection of acid-fast bacteria such as *Mycobacterium tuberculosis*.[111] Ziehl-Neelsen staining is an inexpensive, rapid test with a high positive predictive value; however, it requires large sample volumes and has a low detection rate.[112] Conventional Ziehl-Neelsen staining has a sensitivity of 22.9% and a specificity of 100%, whereas a modified Ziehl-Neelsen staining technique, which requires less sample volume, demonstrates sensitivity and specificity of 100%.[112]

Blood contamination

A significant confounder of CSF collection is blood contamination.[13,17,23,38,48,51] A CSF sample is considered contaminated if there are 10 to 500 erythrocytes/μL or greater.[16,17,20,23,43,47,48] Additional indicators of contamination include components that are not typically present in CSF, specifically the presence of hemoglobin chains,[23,93] particularly at a level of greater than 30 pg/mL,[48] and apolipoprotein B-100.[15,23,93] Blood contamination of CSF may have detrimental effects on the CSF sample and subsequent biomarker analysis, as both serum and plasma contain as great as 400 times the concentration of proteins compared with CSF,[13,16,17,20,23,31,48,56,113] and as a result CSF protein concentration increases approximately 1.1 to 2 mg/dL for every 1000 cells/mm^3 increase in red blood cells.[113,114] Thus blood contamination can lead to some false-positive CSF biomarker measurements.[17,23,38] CSF protein can also be degraded by proteases that are localized in the

TABLE 6.
Cerebrospinal Fluid Parameters for Neurological Diseases

Neurological Disease	Total Protein (g/L)	Glucose Ratio	Lactate (mmol/L)	Cell Count ($\times 10^6$/L)	Typical Cytology	Culture	CSF Color	Opening Pressure (mm H_2O)
Normal values[1,2,14,98]	<0.4-0.45	>0.4-0.6 60% of serum glucose level	<1.0-2.9	<15	No neutrophils; <6 lymphocytes ($\times 10^6$/L); No RBC	Negative	Clear Colorless	100-200
Acute bacterial meningitis[1-5,14,98]	WNL/ increased	WNL/decreased	Increased	>1000	Predominant neutrophils	Positive	Cloudy	>200
Viral meningitis[2,4,5,8,11,14]	WNL/ increased	WNL/decreased	WNL	10-1000	Early: neutrophils increased Late: lymphocytes increased	Negative	Clear to cloudy	WNL/ increased
Fungal meningitis[1,2,5]	Increased	NA	NA	10-500	Predominant lymphocytes	Positive	Clear to cloudy	Increased
Tuberculosis[1,2,4,5]	WNL/ increased	WNL/decreased	NA	50-1000	Early: neutrophils increased Late: lymphocytes increased	Positive for acid-fast bacilli	Cloudy	Variable
Autoimmune polyneuropathy[14]	Increased	WNL	WNL	WNL	NA	NA	Clear to cloudy	WNL
Infections polyneuropathy[99]	Increased	WNL	WNL	Increased	Lymphocytes normal or increased	NA	Clear to cloudy	WNL/ increased
Subarachnoid hemorrhage[2,6,100]	Increased	WNL	WNL	Increased	Erythrocytes, macrophages, siderophages Increased	NA	Bloody with xanthochr-omia	>200
Multiple sclerosis[100]	WNL/mild increase	WNL	WNL	WNL/ increased	Lymphocytes normal or increased	NA	Clear	WNL
Leptomeningeal metastases[101]	Increased	WNL/increased	NA	WNL/ increased	Malignant cells, mononuclears	NA	Bloody with xanthochr-omia	Increased
Leukemia[6]	Decreased	NA	NA	Increased	Blasts comprising >40% of total cells	NA	NA	NA[102-107]

Abbreviations:
CSF = Cerebrospinal fluid
NA = Not applicable
WNL = Within normal limits

TABLE 7.
Cerebrospinal Fluid Biomarkers of Pediatric Diseases

Disease	Biomarker	Notes
Inflammatory CNS diseases	ROS*[,128]	Elevated; contribute to neuroinflammation and disease progression, may indicate severe and rapidly progressive disease or disorder. Antioxidant therapy may decrease incidence of neurological sequelae
	Anti-myelin oligodendrocyte glycoprotein antibodies[108]	Elevated in demyelinating disorders
	T-tau*[,124]	Elevated
	GFAP*[,124]	Elevated
	NFL*[,124]	Elevated
	CXCL-13*[,129]	Elevated in demyelinating disorders
Multiple sclerosis	Intrathecal OCB*[108,125]	Elevated
	IgG*[130,131]	Elevated
	Human TTR[38]	Role of TTR oxidation/oxidative stress
	Secretogranin III[122]	Upregulated at disease onset, indicative of acute inflammation. Downregulated as disease progresses causing axonal loss and degeneration
Optic neuritis	OCB[108]	Presence of OCB is a predictor of disease progression to multiple sclerosis
Lymphocytic hypophysitis	Total protein concentration*[,132]	Elevated
Late-onset hydrocephalus	Total protein concentration*[,34]	Elevated; likely due to an infectious mechanism, typically meningitis
Aicardi-Goutieres syndrome	IFN-α[108]	Elevated
	Neopterin[108]	Elevated
	CSF pleocytosis[108]	Present
Neuropsychiatric systemic lupus erythematosus	OCBs[108]	Elevated
Neuromyelitis optica	NMO-IgG binding[108]	Occurs in 47% of children and is very specific to NMO. Positively correlated with length of spinal cord lesions,[133] associated with relapse[134] and disease progression[135]
	Anti-AQP4 antibody[136]	Elevated. Related to anti-AQP4 astrocytic damage. Associated with severity of NMO and with length of spinal cord lesions
	GFAP[137]	Elevated. Related to anti-AQP4 astrocytic damage. Associated with severity of NMO and with length of spinal cord lesions
	S100 calcium-binding protein B (S100 B)[137]	Elevated
Encephalitides	T-tau, NFL, GFAP, and albumin*[,124]	Combination of these four biomarkers differentiates between progressive and static encephalopathy
	OCBs[108]	Elevated
TBI	NSE*[51,53,138-143]	Elevated. Marker of cellular injury, indicator of severity. No pediatric limitations. Correlated with GOS at 6 months. Higher levels in children with unfavorable outcome

(*continued on next page*)

TABLE 7. (*continued*)

Disease	Biomarker	Notes
	S100B*[22,53,138-140,144]	Elevated. Marker of cellular injury, indicator of severity and indicator of survival time in fatal head injuries. Effective diagnostic marker of hemorrhagic transformation and vasospasm. Not appropriate in children less than two years old due to high normative values. Correlated with PCPC at discharge and 6 months (serum only) and GOS at 6 months
	Heme oxygenase 1(HO-1)*[,145]	Elevated; associated with severity of injury and neurological outcome
	IL-1β*[140,146]	Elevated; correlated with GOS at 6 months Higher IL-1β CSF/serum ratio associated with an increased risk for development of post-traumatic epilepsy following TBI
	IL-4*[,147]	Increase associated with AHT
	IL-6*[53,99,100,115,140,147]	Elevated, proinflammatory cytokine, role in regenerative process. Correlated with GOS at 6 months through the first year
	IL-8*[99,140,148]	Elevated, proinflammatory chemokine, neutrophil chemotactic and activating activity. Associated with in-hospital mortality
	IL-10*[99,115]	Elevated, anti-inflammatory cytokine, associated with age and mortality
	IL-12*[99,147]	Elevated; proinflammatory cytokine, associated with AHT
	MIP-1α*[,99]	Elevated
	VEGF*[53,149]	Elevated, angiogenic cascade initiation
	GFAP*[55,138,140]	Elevated, indicator of CNS injury (serum levels correlated with PCPC and GOS at 6 months)
	NGF*[,140]	Elevated; correlated with GOS at discharge and 6 months
	Beclin 1*[,143]	Elevated
	Sequestosome 1 (or p62)*[,143]	Elevated; correlated with unfavorable outcome at 6 months
	B-cell lymphoma 2 protein*[,150]	Elevated; antiapoptotic protein increased in TBI survivors
	mtDNA*[,151]	Elevated; correlated with neurological outcome
	Mitochondrial heat shock protein 60 (hsp60)*[,101]	Elevated; indicator of severity of injury (GCS at admissions)
	HMGB-1*[140,152]	Elevated; correlated with GOS at 6 months
	Cytochrome c*[140,152,153]	Elevated; proapoptotic protein correlated with GOS at 6 months. Potential biomarker of AHT
	QUIN*[154,155]	Elevated; higher levels in AHT, associated with mortality
	DCX*[,140]	Elevated; correlated with GOS at 6 months
	ET-1*[140,156]	Elevated; vasoconstrictor associated with hypoperfusion following TBI. Correlated with GOS at 6 months
	α-Syn*[140,157]	Elevated; initial early increase followed by a delayed secondary increase. Associated with AHT, younger age, and gender
	F(2)-isoprostane*[141,158]	Elevated; marker of oxidative stress
	Ascorbate*[,158]	Decreased; attributable to free radical oxidation
	Adenosine concentration*[,159]	Elevated; neuroprotective, time and severity dependent
	Glutamate concentration*[,160]	Elevated; highest concentrations often found in abuse victims. Likely a result of hypoxic-ischemic insult. Associated with a rise in adenosine levels
	MBP*[138,161]	Elevated; marker of axonal damage; prolonged increase of several days following TBI
	Sulfonylurea receptor-1*[,162]	Elevated; correlated with CT edema, decreasing trend between 48 and 72 hours associated with improved cerebral edema and outcome
Status epilepticus	T-tau*[51,124,163,164]	Elevated, indicates cortical axonal engagement. Highest concentration when patients in status epilepticus. Potential indicator of severity and prognosis in adults
	Ubiquitin carboxy-terminal hydrolase-L1[165]	Elevated following recurrent epileptic seizures in adults
	GFAP*[51,124,166]	Elevated, indicates astrocytic injury. Primarily observed in prolonged seizures or patients in status epilepticus
	NSE*[51,167,168]	Elevated (specifically observed in medically refractory status epilepticus not cryptogenic or idiopathic or febrile seizures), marker of neuronal damage
	5-HIAA and HVA*[,169]	There is not a clear correlation between 5-HIAA and HVA and status epilepticus. Some reports have shown that they can be elevated, whereas others show decrease or no changes
Subarachnoid hemorrhage	Total protein concentration[25]	Elevated
	Oxyhemoglobin[20,25,119]	Elevated. Will persist in CSF for days or be converted to methemoglobin. Produced *in vitro* and *in vivo*
	Bilirubin[20,25,119,120]	Elevated. Ideal time period for collection is > 6-12 hours. Will persist in CSF for up to a month. Produced *in vivo* only
	GFAP[170]	Altered GFAP washout patterns noted between survivors and nonsurvivors. Secondary GFAP peak associated with additional ischemic insult and provides prognostic value in survivors
	20-Hydroxyeicosatetraenoic acid[171]	More likely to have mortality, clinical neurological decline, and modified Rankin scores at 3 and 12 months.
	IL-6[168]	Elevated; correlated with increased severity
	Tumor necrosis factor-α[172]	Elevated; correlated with increased severity
	Arginine-vasopressin[173]	Decreased; correlated with poor outcome
	Oxytocin[173]	Decreased
Pediatric neurotransmitter disease	Homovanillic acid*[,49]	Altered metabolite levels are nonspecific indicators of various neurotransmitter disorders involving the dopaminergic and serotonergic metabolic processes and must be evaluated alongside the clinical picture
	5-HIAA*[,49]	
	3-O-Methyldopa*[,49]	
	Tetrahydobiopterin*[,49]	
	Neopterin*[,49]	

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TABLE 7. (*continued*)

Disease	Biomarker	Notes
Infections	ET-1[*,174]	Elevated in children with enterovirus-71 encephalitis with NPE
	Neopterin[*,175]	Associated with inflammatory lesions in enterovirus-A71-associated encephalitis
	Apolipoprotein[*,35]	Marker of invasive bacterial infections
	S100 B[*176,177]	Elevated in children with bacterial meningitis. May contribute to severity and neurological complications In tuberculous meningitis the highest lumbar concentrations and increasing trends as well as the highest ventricular concentrations are associated with mortality and 6-month outcome
	IL-6[*178,179]	Elevated in children with RSV-associated encephalopathy and correlated with PCPC score. Predictor of neurological complications in HHV-6 associated acute encephalopathy
	Brain-derived neurotrophic factor[*,178]	Elevated in children with RSV-associated encephalopathy and correlated with PCPC score
	Nitric oxide and lipid peroxide[*,176]	Elevated in children with bacterial meningitis. Mediators of oxidative stress. May contribute to severity and neurological complications
	Superoxide dismutase and total thiol[*,176]	Elevated in children with bacterial meningitis. Mediators of antioxidation. May contribute to severity and neurological complications
	Intrathecal OCB[*51,108,180,181]	Present in acute or chronic encephalitis. Clonal IgG binds with high affinity to infectious agent. Detected in up 100% patients with herpes simplex encephalitis and human immunodeficiency virus encephalitis
	Procalcitonin[*,182]	Marker of bacterial versus aseptic meningitis
	Neurofilament (heavy subunit)[183]	Elevated in subacute sclerosing panencephalitis. Marker of disease progression
	Soluble tumor necrosis factor receptor 1[183]	Elevated in subacute sclerosing panencephalitis.
	T-Tau[*118,184]	Elevated in patients with serious CNS infection, including HHV-6-associated acute encephalopathy
	CXCL-13[*,185]	Elevated in Lyme neuroborreliosis, consider as complementary diagnostic tool
Hydrocephalus	T-Tau[*,118]	Elevated. Marker of axonal and neuronal damage
	Cleaved-tau[*,186]	Elevated in children with hydrocephalus who display signs of increased intracranial pressure and require shunt placement or revision. Marker of axonal damage. Related to age
	Total Protein[*,34]	Elevated in LOH
	5-HIAA[*,187]	Elevated, indicative of increased metabolism and/or absorption of serotonin associated with ICP alterations and decreased clearance due to obstruction resulting from increased ICP
	Soluble Fas (sFas)[*34,188]	Regulation of apoptosis. Elevated in PHH and SB/HC
	Amyloid precursor protein[*189,190]	Elevated in PHH. Indicator of axonal injury or stretch. Associated with ventricular size
	L1 cell adhesion molecule[*,190]	Elevated in PHH
	NCAM-1[*189,190]	Elevated in PHH. Correlated with ventricular size
	Stem cell factor[*,34]	Elevated in SB/HC. Involved in cell survival, proliferation, and differentiation
	Hepatocyte growth factor[*,34]	Elevated in PHH. Involved in neuronal repair, recovery, survival, and maturation. Present in hydrocephalus regardless of etiology
	VEGF[*34,191]	Elevated in hydrocephalus, specifically PHH and SB/HC. Regulates angiogenesis
	IL-6[*,34]	Elevated in LOH and SB/HC
	NGF[*,192]	Intrathecal production elevated. Role in neuronal differentiation and survival
	Neurotrophin-3[*,192]	Elevated. Role in neuronal differentiation and survival
	GFAP[*,94]	Elevated in PHH. Indicates damage to astrocytes and ependyma
	MBP[*,94]	Elevated in PHH and SB/HC. Indicates damage to oligodendrocytes and myelin
	IL-18[*,193]	Elevated in human neonatal human neonatal HPHC, thought to contribute to diffuse white matter injury
	IFN-γ[*,193]	Elevated in human neonatal HPHC, thought to contribute to diffuse white matter injury
Pediatric brain tumors	T-tau[*,118]	Elevated. Marker of axonal and neuronal damage
	Cleaved-tau[*,194]	Elevated (400-fold). Marker of axonal and neuronal damage
	Prostaglandin D2 synthase[*,91]	Decreased (sixfold) in medullablastoma. Host response to tumor
	Leu-Val-Val- and Val-Val-hemorphin-7[*,92]	Decreased in patients with brain tumor and in postoperative patients with residual tumor or metastases. Potential prognostic indicator. When obtained from cisterna magna can indicate biologic aggressiveness of tumor
	Apolipoprotein A-II[*,116]	Elevated. Correlated with increased albumin concentration, likely indicative of blood-brain barrier dysfunction
	Basic fibroblast growth factor[*,195]	Elevated in patients with brain tumors
	Cyclophilin A[*,196]	Elevated in DIPG. Found in variety of tumor types. Associated with malignant transformation and decreased survival
	Dimethylarginase 1[*,196]	Elevated in DIPG. Found in a variety of tumor types. Associated with tumor angiogenesis
	IGF[*117,197,198]	IGFBP-2 and IGFBP-3 elevated in patients with medullablastomas and ependymomas. IGFBP-2 indicates poor prognosis in patients with medullablastoma IGFBP-3 protease activity increased in tumors with high-grade histologic malignancy. Potential marker of residual disease in medullablastomas. Lack of detection in serum suggests local production by CNS tumor tissue or malignant cells in CSF IGFBP-5 and IGF-II elevated in ependymomas
	Osteopontin[*199,200]	Elevated in patients with atypical teratoid/rhabdoid tumor. Associated with aggressiveness and poor outcome
	Placental alkaline phosphatase[*,201]	Elevated in intracranial germinomas
	Polysialylated isoform of neural cell adhesion molecule[*,202]	Elevated. Medullablastoma cell surface marker. Possible role in metastasis or tumor progression

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TABLE 7. (*continued*)

Disease	Biomarker	Notes
Sudden infant death syndrome	IL-6*[87,203,204]	Elevated. Increased IL-6 levels in conjunction with altered IL-6 receptor expression in the arcuate nucleus thought to play interactive role. Scatter of levels may indicate a mechanism more similar to infectious process and one more similar to trauma. Correlated with infectious indicators including increased number of IgA immunocytes in laryngeal mucosa
	VEGF*[,205]	Elevated. VEGF stimulates angiogenesis in response to hypoxia and is suggestive of at least one hypoxic event before death
Depression	Apolipoprotein E[206]	Decreased. Potential link to metabolic syndrome, which may predispose individual to major depression
	Cystatin C[206]	Decreased. Cysteine proteinase inhibitor, may indicate cognitive dysfunction
	PEDF[206]	Elevated. PEDF a neutrophilic glycoprotein with a potential compensatory mechanism for endogenous proapoptotic stress
	Prostaglandin-D synthase[206]	Decreased. Potential role in sleep disturbances
PD	Fms-like tyrosine kinase 3 ligand[207]	Differentiates between PD and multi-system atrophy (99% sensitivity, 95% specificity)
	Fractalkine/$A\beta_{1-42}$[207]	Marker of severity and progression
	Total biopterin[208]	Decreased but to a lesser extent in juvenile (below age of 40) Parkinson compared with typical. Related to age at onset and indicates the amount of degeneration in nigrostriatal dopaminergic nerve terminals
	T-Tau[209]	Increased over time; stable in early phases, indicator of disease severity
	Phosphorylated tau[209]	Increased over time; rapid increase indicative of more rapid motor and cognitive decline
	α-syn[209]	Increased over time; stable in early phases, indicator of disease severity
	NFL[209]	Increased over time
	Chitinase-3-like protein 1 (YKL-40)[209]	Increased over time, rapid increase indicative of more rapid cognitive decline

Abbreviations:
5-HIAA = 5-Hydroxyindoleacetic acid
α-Syn = α-Synuclein
AHT = Abusive head trauma
AQP = Aquaporin 4
CNS = Central nervous system
CSF = Cerebrospinal fluid
CT = Computed tomography
CXCXL-13 = Chemokine ligand 13
DCX = Doublecortin
DIPG = Diffuse intrinsic pontine glioma
ET-1 = Endothelin-1
GFAP = Glial fibrillary acidic protein
GCS = Glasgow Coma scale
GCS = Glasgow Outcome scale
HHV-6 = Human herpesvirus-6
HMGB = High-mobility group box 1
HPHC = High-pressure hydrocephalus
HVA = Homovanillic acid
ICP = Intracranial pressure
IFN-α = Interferon-α
IL-1β = Interleukin-1β
IGF = Insulin-like growth factor
IGFBP = IGF-binding protein
IgG = Immunoglobulin G
LOH = Late-onset hydrocephalus
MBP = Myelin basic protein
MIP = Macrophage inflammatory protein
mtDNA = Mitochondrial DNA
NCAM = Neural cell adhesion molecule-1
NFL = Neurofilament light
NGF = Nerve growth factor
NMO = Neuromyelitis optica
NPE = Neurogenic pulmonary edema
NSE = Neuron-specific enolase
OCB = Oligoclonal bands
PCPC = Pediatric performance category scale
PD = Parkinson disease
PEDF = Pigment epithelium-derived factor
PHH = Posthemorrhagic hydrocephalus
ROS = Reactive oxygen species
RSV = Respiratory syncytial virus
SB/HC = Spina bifida with hydrocephalus
T-Tau = Total tau
TTR = Transthyretin
VEGF = Vascular endothelial growth factor
* Indicates biomarkers with pediatric data

TABLE 8.
Metabolomics and Lipidomics for Biomarker Discovery

Biomarker(s)	Diseases of Interest	Potential Limitations
Metabolomics		
Homovanillic acid[49,212]	Tetrahydrobiopterin (BH4) deficiency disorders[49,213]	Sample volumes[93]
5-Hydroxyindoleacetic acid[49,214]	Tyrosine hydroxylase deficiency[212]	Variability in chemical structure of metabolites[93]
3-O-methyldopa[49]	Aromatic L-amino acid decarboxylase deficiency[214]	Overall lower metabolite concentration in CSF than blood[93]
Tetrahydrobiopterin[49]		Specific methodology for identification of neurotransmitters and steroids (i.e., gas chromatography and electron capture negative chemical ionization mass spectrometry)[215]
Neopterin[49]		
Lipidomics		
Platelet-activation factor acetyl hydrolase[210]	Inflammatory disease processes (e.g., ischemic stroke, diabetes mellitus, and systemic lupus erythematosus)[210]	Tandem liquid-gas chromatography and mass spectrometry[210]
Cytochrome P450[101]	Cholesterol and steroid biosynthesis (influence on brain growth and development)[210]	Free radical chemical analysis of oxidized lipids[210]
F2-isoprostane[138]	TBI and secondary CNS damage[138]	
Cardiolipin[138]		

Abbreviations:
CSF = Cerebrospinal fluid
CNS = Central nervous system
TBI = Traumatic brain injury

plasma.[15,38] The degenerative effects of WBCs on the observed proteome as well as other metabolites and amino acids are observed even in the absence of erythrocyte contamination and contribute to sample degradation.[38,57]

Traumatic LPs resulting in blood contamination of the CSF sample occur with an incidence of 14% to 72%.[17,20,23,38,113,114] A traumatic LP may be due to the puncture of the venous plexus or vessels found in the ventral epidural space.[20,54] Elevated protein levels in CSF samples contaminated by traumatic LP are difficult to analyze.[113] Recommendations to limit the influence of blood contamination include expedited transport to laboratory on ice followed by centrifugation, and immediate freezing at −80°C.[17,20,23,26,36,38,48,51,54,56,57,62,115-118]

Spectrophotometric analysis

Spectrophotometric analysis of CSF is able to identify peaks of oxyhemoglobin, methemoglobin, and bilirubin and is a useful diagnostic measure for patients with a subarachnoid hemorrhage (SAH).[20,119] Bilirubin is produced *in vivo* and as such can be used to distinguish between a traumatic LP and an intracranial bleed.[20] To avoid the probability of a false-positive subarachnoid hemorrhage diagnosis the last fraction of CSF collected should be used for spectrophotometry.[20,119] The ideal time frame for sampling to determine the presence of bilirubin is six to greater than 12 hours after initial onset but can be detected up to one month post-onset.[14,20,25,26,119] After sampling, bilirubin degrades expeditiously in light when compared with dark,[120] mandating rapid processing of CSF samples as soon as possible and protection of the CSF samples from light.[119,120] A blood sample should be collected concurrently to determine serum bilirubin and total protein concentrations for comparative analysis.[20,26,119] Caution should be taken when analyzing CSF in neonates as CSF xanthochromia is relatively common in the first month of life (28%).[121] The frequency of CSF xanthochromia is negatively correlated with age.[121] Delivery history should be considered as there is an association of CSF xanthochromia with maternal history of labor and birth method.[113,121]

Biomarkers of pediatric diseases

Laboratory-based analysis of CSF proteins or peptides can provide insight into possible biomarkers of pediatric diseases, which may allow for new and potentially earlier diagnostic and treatment options.[17,18,34,38,51,91,92,95,108,122-126] A biomarker is an indicator of the current biologic state,[38] and biomarker research is expanding our ability to diagnose and expedite treatment for a number of diseases. A good biomarker must be able to reliably differentiate between normal and disease states as well as differentiate between different diseases.[127] Research conducted for the identification of possible biomarkers for a number of pediatric diseases and disorders is expanding exponentially. Lack of sufficient CSF sample collection is a major hurdle in the research of neurological diseases, leading to insufficient powering of research studies.[17]

Some diseases have specific biomarkers such as *N*-methyl-D-aspartate receptor encephalitis, whereas nonspecific biomarkers such as oligoclonal bands, neopterin, and ferritin may be utilized to indicate CNS inflammation as well as demyelination, infection, metabolic stress, neuronal injury, and vascular injury.[20,108,125] Pediatric CSF biomarker research is focused on early-onset multiple sclerosis, juvenile-onset Parkinson disease, amyotrophic lateral sclerosis, pediatric brain tumors, pediatric neurotransmitter diseases, and traumatic brain injury (Table 7). Although proteomics is the more prominent field of CSF biomarker research, alterations in CSF metabolite and lipid concentrations can provide important supplementary information.[31,93,210]

Metabolomics and lipidomics

Metabolomics, the study of chemical compounds produced by way of specific cellular processes,[211] is utilized for the identification of pediatric disorders of neurotransmitters and inborn errors of metabolism.[49] Abnormal metabolite profiles have been suggested as potential biomarkers and may indicate a number of disorders (Table 8).[49] Owing to the sensitive nature of certain metabolites (i.e., tetrahydrobiopterin) samples require immediate freezing on dry ice at bedside or collection into an antioxidant mixture.[49]

Lipidomics is the study of structure, biosynthesis, and function of lipids.[210] Lipids compose approximately half of the dry mass of the brain and contribute to various brain functions including membrane composition, signal transduction, and messenger functions.[210] As such, changes in lipid metabolism and resultant lipid concentrations may indicate the presence of several disease processes (Table 8).[210] Lipidomics provides detailed information regarding lipid composition in CSF, whereas concurrent proteomic analysis can help to understand the mechanisms by which changes in the lipidome occur (i.e., altered protein concentrations or levels of protein activation).[210]

Conclusion

This review summarizes pertinent issues regarding collection, processing, and storage of pediatric CSF. The collection, processing, and storage of CSF are key bio-banking steps allowing for investigations on CNS-related diseases. Adherence to ethical standards, while maintaining sample integrity, is critical in translational research. CSF investigations will contribute to new diagnostics and therapeutics in childhood disease.

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