MONITORING PATHOPHYSIOLOGY IN THE INJURED BRAIN

EDITED BY: Eric P. Thelin, David W. Nelson, Adel Helmy and Niklas Marklund
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MONITORING PATHOPHYSIOLOGY IN THE INJURED BRAIN

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Abstract brain wave concept on blue background technology.

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Pathophysiological processes in brain-injured patients can be assessed with an array of methods, with a goal to identify potentially deleterious events, guide treatments and avoid further deterioration. This eBook provides an in-depth exploration into different aspects of neurocritical care monitoring and how new tools and strategies may be utilized to improve patient outcomes.

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EDITORIAL
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Editorial: Monitoring Pathophysiology in the Injured Brain

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Editorial on the Research Topic

Monitoring Pathophysiology in the Injured Brain

INTRODUCTION

Brain injuries can be caused by, for instance, spontaneous hemorrhages, thromboembolic incidents or traumatic events, and are considerable sources of morbidity and mortality (1). Brain injuries commonly result in immense socioeconomic consequences due to the acute as well as persisting neurological deficits (2). To date, there are currently few pharmacological therapies of proven clinical benefit targeting the underlying pathophysiology occurring in the aftermath of subarachnoid hemorrhage (SAH) (3), stroke (4), and traumatic brain injury (TBI) (5). Although these brain injuries are exceedingly heterogeneous, some common pathophysiological phases may be identified. At disease onset, the primary ictus may cause initial neuronal, glial, and vascular injury, which is then followed by complex pathophysiological responses. The initial tissue injury is then exacerbated by secondary insults, occurring in the vulnerable brain during the first post-injury period (6). The secondary injury cascades include among others neuroinflammation, energy failure, hypoxia, and inadequate cerebral perfusion (7). By monitoring multiple intracerebral as well as systemic parameters across a range of modalities, the time course of these detrimental secondary injury cascades can be ascertained. Importantly, knowledge acquired from monitoring and investigation of these injury processes is expected to facilitate the future development of novel treatment strategies. Recently, improved intracranial monitoring was stressed as one of the key areas of research by a commissioned article devoted to TBI in a Lancet Neurology editorial (8).

In this Research Topic, a number of authors from several key centers of excellence worldwide have shared their knowledge on the monitoring of acute brain injuries. These contributions provide updated knowledge of the pathophysiology of TBI and other acute brain injuries, as well as of refining patient management strategies. The overall goal of improving patient outcomes by the detection of deleterious secondary injury processes occurring in the injured brain.

NEUROIMAGING

- Stovell et al. reviewed the monitoring of brain metabolism using magnetic resonance spectroscopy (MRS) in acute TBI. While this technique is still in its infancy, it holds much promise as increasing evidence suggests that deranged metabolism is a major exacerbating factor following brain injury, especially in relation to mitochondrial dysfunction. The MRS technique shows potential for the evaluation of specific brain regions where tissue fate may be analyzed granularly in both acute and chronic phases of brain injury.

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- Rostami et al. evaluated the correlation between cerebral blood flow and metabolism in patients following SAH monitored using Xenon-CT in combination with cerebral microdialysis (CMD). They observed that reduced global blood flow the first 3 days post-SAH was significantly associated with brain metabolism monitored using CMD, specifically high levels of lactate indicating anaerobic metabolism. These results suggest that monitoring of cerebral blood flow and brain neurochemistry could aid physicians and guide treatments, such as optimizing cerebral perfusion pressure (CPP) and adjusting oxygen and glucose substrate levels in SAH patients.

COAGULATION

- Lindblad et al. evaluated multiple electrode aggregometry (Multiplate®) used to assess platelet functions in NICU-treated TBI patients. Although platelet count may be normal, several studies have shown that they do not function properly following TBI. Thus, the primary hemostasis may be altered, specifically by an impaired response of the arachidonic acid (ASPI) receptor. The authors could show that 65% of the included TBI patients had abnormally low ASPI values. This was associated with a more unfavorable outcome, although interestingly not with hemorrhagic progression. These results highlight the need for future research and validated methods of platelet assessment for patients not on platelet anti-aggregation therapies.

CLINICAL INVESTIGATIONS

- Marklund evaluated the current role of the neurological wake-up test (NWT) in neurocritical care monitoring, reviewing the available literature. While intracranial pressure (ICP) and CPP monitoring as well as serum markers indicate that the NWT could induce a mild stress response including increased ICP and the release of stress-related hormones, the consequences of these are not fully understood. While qualitative prospective studies concerning its safety are lacking, the NWT remains an important monitoring tool in selected TBI and SAH patients, preferably in combination with multimodal monitoring.

CEREBRAL MICRODIALYSIS

- Carteron et al. report on how CMD has allowed them to individualize neurocritical care therapy in TBI and SAH patients. Specifically, CMD may help guide optimization of CPP, blood transfusions, glucose infusions, and brain tissue oxygenation targets. Newer markers include electrolytes, markers of oxidative stress, or endothelial proteins, which may also be measured by CMD in order to better predict outcome or guide treatment.
- Helbok et al. conducted a systematic review on the clinical use of CMD in patients with aneurysmal SAH, focusing on secondary brain injury and clinical outcome. They found that the metabolic changes detected by CMD were associated with early and delayed secondary brain injuries and suggested that CMD be used in conjuncture with other monitoring modalities. In summary, while CMD in SAH is less studied than in TBI, it is an emerging area that might establish itself as a future standard monitor of SAH patients, awaiting additional investigations and multi-center trials.

BIOMARKER MONITORING

- Ercole et al. review and summarize several different technologies to monitor molecular signals in TBI. Mass spectroscopy as well as optical spectroscopy also hold a potential to unravel the *in vivo* molecular signatures in TBI patients. Altogether, by introducing an "-omics" approach in TBI, it will be possible to gain further understanding of the systems biology involved.
- Posti et al. reviewed the field of metabolomic monitoring as a diagnostic tool in severe TBI, by investigating different metabolites (fatty acids, amino acids, as well as sugar derivates), and how they are associated with injury severity and clinical outcome. This is a promising field although larger prospective trials are necessary to establish better thresholds and specific metabolic patterns associated with brain injury and patient outcome.
- Thelin et al. analyzed the current literature, focusing on some of the most commonly studied biomarkers including S100B, neuron-specific enolase (NSE), glial fibrillary acidic protein (GFAP), ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), and neurofilament light (NF-L) in order to review serum level dynamics of these proteins following TBI. In aggregate, shorter half-lives were seen for S100B and UCH-L1 in contrast to NSE, GFAP, and especially NF-L that exhibit increased levels for prolonged periods of time post-injury. These differences should be taken into account when assessing the capability of these biomarkers to predict outcome and monitor secondary pathologies in TBI patients.
- Tsitsopoulos et al. reviewed possibilities for short- and long-term monitoring of axonal injury in TBI. Biomarkers, specifically NF-L, is mentioned as a potential diagnostic candidate, as it has been found enriched in myelinated axons and seen to be elevated in serum in patients with radiologically verified DAI. Novel MRI tools are increasingly used for the detection and progress of axonal injury.

NEUROINFLAMMATION

- Zeiler et al. reviewed the literature on CMD and CSF cytokines in both TBI and SAH patients. They could conclude that there is an association with elevated levels of certain cytokines, among others, interleukin-6 and tumor necrosis factor alpha, and long-term functional outcome. In summary, cytokines as mediators of inflammatory activity can be monitored in the extracellular fluid by CMD and in CSF and appear to be associated with secondary injury pathophysiology and clinical outcome in both TBI and SAH.
- Thelin et al. reviewed several modalities that are used to monitor the neuroinflammatory response following acute brain injury, focusing on TBI and SAH. In summary, the authors suggest a multimodal monitoring approach in order

to improve the understanding of the neuroinflammatory response following acute brain injury to determine its role for tissue as well as patient outcome.

- Vink et al. summarize the role of Substance P in the secondary pathophysiology of TBI, reviewing its role in the neurogenic inflammation and association to ICP increases as observed in several of their studies. In summary, the role of substance P in neurogenic inflammation following TBI is increasingly understood and opens up potential future therapeutic targets for patients suffering from acute brain injury.

TREATMENT STRATEGIES

- Zoerle et al. address in their review how to rethink the concept of neuroprotection. They suggest that different aspects of TBI care need to be refined and related to TBI pathophysiology, thus requiring individualized care in part guided by advanced monitoring approaches. These monitoring tools could prompt the clinicians to earlier detection of signs that may need treatment. By using these improved neurocritical care interventions, more adequate neuroprotection might be achieved.

MONITORING STRATEGIES

- Nordström et al. have written a summary on aspects of the physiological and biochemical foundations of NCC treatment strategies. Here, they address the basic concepts of NCC with focus on the key aspects that form the base for the Lund concept, including intracapillary hydrostatic pressure modulation, fluid therapies, and the implementation of brain tissue oxygen- and CMD monitoring.
- Grände critically evaluates the Lund concept, 25 years after its introduction. He focuses his review on temperature, ventilation, nutrition, osmotherapy, decompressive craniectomy, and sedation management. Following the introduction of more advanced monitoring techniques such as CMD, brain tissue oxygen monitoring, and other ICP- and CPP-guiding protocols, many current NCC strategies and targets have gradually approached several of those comprising the Lund concept.
- Figaji reviewed how treatment and monitoring of TBI and spinal cord injury differs between adults and children. As especially younger children have different anatomy and physiology than adults, there are several aspects, which need to be considered by the treating physician. One of the main findings of this literature review is the lack of suitable studies focusing primarily on the pediatric CNS following acute brain and spinal injury. This lack of knowledge could lead to adult thresholds and ranges for intracranial monitoring parameters being used in many pediatric situations, which might be inappropriate or even harmful to this patient population.

CONCLUSION

In this research topic, many aspects of the current knowledge and possible future direction of multimodal monitoring in TBI patients are reviewed. Commonly, only limited aspects of the underlying pathophysiology in acute brain injury can and are monitored in the clinical setting. Introducing additional monitoring modalities including parameters such as inflammation markers, multiple metabolites, and a more granular evaluation of structural injuries, a more comprehensive understanding of the evolution of TBI pathology and ongoing secondary injury processes may emerge in the near future. This will require continued large research efforts, but is expected to facilitate development of novel therapeutic options, personalized medicine, and treatment strategies for patients suffering from acute brain injury.

AUTHOR CONTRIBUTIONS

ET, DN, AH, and NM confirm being the sole contributors of this work and approved it for publication.

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REVIEW
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Assessing Metabolism and Injury in Acute Human Traumatic Brain Injury with Magnetic Resonance Spectroscopy: Current and Future Applications

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Traumatic brain injury (TBI) triggers a series of complex pathophysiological processes. These include abnormalities in brain energy metabolism; consequent to reduced tissue pO_2 arising from ischemia or abnormal tissue oxygen diffusion, or due to a failure of mitochondrial function. *In vivo* magnetic resonance spectroscopy (MRS) allows non-invasive interrogation of brain tissue metabolism in patients with acute brain injury. Nuclei with "spin," e.g., ^{1}H, ^{31}P, and ^{13}C, are detectable using MRS and are found in metabolites at various stages of energy metabolism, possessing unique signatures due to their chemical shift or spin–spin interactions (J-coupling). The most commonly used clinical MRS technique, ^{1}H MRS, uses the great abundance of hydrogen atoms within molecules in brain tissue. Spectra acquired with longer echo-times include *N*-acetylaspartate (NAA), creatine, and choline. NAA, a marker of neuronal mitochondrial activity related to adenosine triphosphate (ATP), is reported to be lower in patients with TBI than healthy controls, and the ratio of NAA/creatine at early time points may correlate with clinical outcome. ^{1}H MRS acquired with shorter echo times produces a more complex spectrum, allowing detection of a wider range of metabolites. ^{31}P MRS detects high-energy phosphate species, which are the end products of cellular respiration: ATP and phosphocreatine (PCr). ATP is the principal form of chemical energy in living organisms, and PCr is regarded as a readily mobilized reserve for its replenishment during periods of high utilization. The ratios of high-energy phosphates are thought to represent a balance between energy generation, reserve and use in the brain. In addition, the chemical shift difference between inorganic phosphate and PCr enables calculation of intracellular pH. ^{13}C MRS detects the ^{13}C isotope of carbon in brain metabolites. As the natural abundance of ^{13}C is low (1.1%),^{13}C MRS is typically performed following administration of ^{13}C-enriched substrates, which permits tracking of the metabolic fate of the infused ^{13}C in the brain over time, and calculation of metabolic rates in a range of biochemical

pathways, including glycolysis, the tricarboxylic acid cycle, and glutamate–glutamine cycling. The advent of new hyperpolarization techniques to transiently boost signal in ^{13}C-enriched MRS *in vivo* studies shows promise in this field, and further developments are expected.

Keywords: ^{1}H MRS, ^{31}P MRS, ^{13}C MRS, trauma, traumatic brain injury, energy metabolism, biomarker

INTRODUCTION

Metabolic Dysfunction in Traumatic Brain Injury (TBI)

Traumatic brain injury is the commonest cause of death and disability in young adults in the developed world and is a significant demand on resources (1). If a person survives the initial traumatic insult a series of pathophysiological processes occur causing further damage to the brain that results in greater disability and even death. These include raised intracranial pressure (ICP), cerebral hypoperfusion, generalized hypoxia, hypoglycemia, neuroinflammation, and metabolic dysfunction. Metabolic dysfunction describes the brain relying on glycolysis (despite the presence of oxygen) as a rapid but inefficient means of synthesizing adenosine triphosphate (ATP)—so generating much less ATP per mole of glucose consumed than if the pyruvate produced by glycolysis feeds into mitochondrial metabolism. It is often ascribed to a failure of mitochondrial function (2, 3). Due to advances in neurointensive care and multimodality monitoring, gross hypoxia and hypoperfusion are generally avoided in patients, and raised ICP is identified and managed. The monitoring, interpretation and treatment of brain metabolic dysfunction and neuroinflammation are more challenging.

"Normal" energy metabolism of the human brain consists of a complex interaction of multistep processes with trafficking of metabolites between different cells types. In each section of our review (^{1}H, ^{31}P, and ^{13}C), we describe the pathways relevant to the technique, and for review see Ref. (4, 5). It should be noted that normal human brain metabolism remains a subject of research and is still not fully understood, but glucose is invariably considered the principal metabolic fuel for the brain. A simplified schematic of major energy pathways in the brain is shown in **Figure 1**. After uptake into the brain, most of the glucose is metabolized *via* glycolysis into two molecules of pyruvate, with a net production of two molecules of ATP and two molecules of NADH in the process. A smaller proportion of glucose is metabolized *via* the pentose phosphate pathway (PPP). The PPP is a complex detour starting from glucose-6-phosphate (hence its alternative name "hexose monophosphate shunt") bypassing some of the steps of glycolysis in the metabolism of glucose. The PPP does not require molecular oxygen, and it does not consume or produce ATP. During the PPP, the first carbon of glucose is lost as CO_2, $NADP^+$ is reduced to NADPH, and various intermediates are produced, including ribose-5-phosphate used in the synthesis of nucleotides and nucleic acids. NADPH participates in reductive reactions such as synthesis of fatty acids and the reduced form of glutathione, a cofactor for glutathione peroxidase. Thus, the PPP has been suggested to play a protective role after TBI, promoting synthesis of molecules for tissue repair and combatting oxidative stress. Therefore, the PPP sacrifices some of the carbon of glucose for the sake of tissue repair. The PPP ultimately rejoins the glycolysis mainstream, and pyruvate may then be incorporated into the tricarboxylic acid (TCA) cycle in cell mitochondria after conversion to acetyl-CoA, where it is metabolized through eight steps, generating three molecules of NADH, one $FADH_2$ and a molecule of GTP. $FADH_2$ and NADH drive the electron transport chain at the mitochondrial membrane, producing ATP from adenosine diphosphate (ADP) by oxidative phosphorylation in the presence of oxygen. ATP is the fundamental molecule of chemical energy in humans and is used to drive cellular reactions and machinery, being converted back to ADP and inorganic phosphate (Pi) in the process. As an alternative to mitochondrial metabolism, pyruvate may stay in the cytosol and be converted to lactate [by the action of lactate dehydrogenase (LDH)], recycling the NADH produced in glycolysis back to NAD^+, so allowing glycolysis to continue. The conversion (chemically, an oxidation) of NADH to NAD^+ in the cytosol can also be accomplished by the action of the electron transport chains of mitochondria, if operational. As NADH

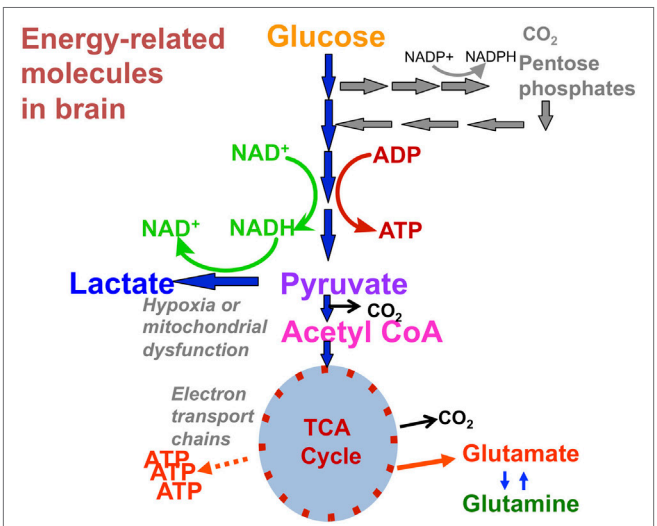

FIGURE 1 | Simplified schematic of major energy pathways in the brain includes glycolysis, which takes place in the cytosol and produces pyruvate, which enters mitochondria and is converted into acetyl-CoA that enters the tricarboxylic acid (TCA) cycle. Alternatively, pyruvate can stay in the cytosol and is converted into lactate that is exported out of the cell. The pentose phosphate pathway takes place in the cytosol and is an alternative pathway that can be upregulated after injury; it is an important source of NADPH used to produce the reduced form of glutathione (GSH) for preventing oxidative stress. This figure was originally published by Carpenter et al. (4). © 2014 The Authors. Published by Elsevier B.V. Open Access under a CC–BY license.

cannot itself cross the mitochondrial membrane, the requisite hydrogens and electrons are transferred indirectly by "shuttle" mechanisms. For more information on the above biochemical pathways in the context of the brain, see (6–8).

Studies using a range of techniques have shown that the human brain will take up and directly metabolize alternative fuels such as lactate, acetate, beta-hydroxybutyrate and ketone bodies (4, 5, 9). Shuttling of fuels is also thought to occur between different cell types: the astrocyte-neuron-lactate shuttle hypothesis suggests that astrocytes take up glucose from the blood supply, convert it to lactate, then feed that to their surrounding neurons for conversion back to pyruvate and then metabolism by the TCA cycle (10). A further neuronal–astrocyte coupling is the glutamate (Glu)–glutamine (Gln) cycle, whereby TCA cycle intermediate α-ketoglutarate is converted to Glu for neurotransmission. After Glu is released it is taken up by local astrocytes, converted to Gln, and then fed back to the neurons for conversion back to Glu and thence to α-ketoglutarate, which can reenter (termed anaplerosis) into the TCA cycle, or else Glu can be released for further neurotransmission (5).

Disruption and changes to human brain metabolism following acute severe TBI depend on injury severity and how long after the injury occurred. In the acute phase, a depression of the metabolic rate of glucose and a fall in oxygen consumption is generally reported (11). Brain extracellular lactate may rise following TBI (3, 6), but because lactate is a recognized brain fuel, changes to its absolute concentration are difficult to interpret. More useful is the ratio of lactate/pyruvate as the exchange of these species are at fast equilibrium, directly proportional to the ratio of $NADH/NAD^+$ (redox state of the cell) which correlates with outcome following TBI (3, 12).

The metabolic state of the brain and markers of degree of injury can be interrogated with magnetic resonance spectroscopy (MRS), microdialysis, positron emission tomography (PET), and arterio-venous (AV) difference measurements of metabolites. The limitations of microdialysis are its invasive nature involving insertion of intracerebral catheters, its sampling is confined to the extracellular compartment and its highly focal nature means that generalization to the rest of the brain is uncertain. PET is relatively less invasive and reflects the intracellular and extracellular compartments of the brain, but involves the exposure of patients to intravenously injected radioactive (short half-life) ligands, and is usually combined with CT or MRI to enable optimal localization of the PET signal. AV difference studies are invasive and have become less convenient as jugular bulb venous catheters are nowadays not routinely used in the management of patients with acute TBI (2).

Prognosticating in severe TBI can also be difficult. Patient age, neurological status at presentation, and cardiovascular stability are known to correlate statistically with outcome at 6 months (1) but are unable to reliably predict outcome in every individual case. Other biomarkers for prognostication include ICP and the marker of metabolic dysfunction, L/P ratio, which is measured by microdialysis (2). Further prognostic markers that can strengthen existing predictive models of outcome will allow more informed decisions from relatives and clinicians for ceilings of treatment and standardization of injury severity in research studies and clinical audit (1, 2).

In vivo MRS allows interrogation of key aspects of brain metabolism and has prognostic value. It is non-invasive, does not involve ionizing radiation, and measures metabolites from whole brain tissue; both the extracellular compartment and also the intracellular compartment [which contributes 80% of total brain volume (13, 14)] of the region selected. Currently, its use is limited to research but this review will discuss the changes in brain metabolites and biomarkers measured by *in vivo* MRS following acute severe TBI, its potential for clinical monitoring to guide treatment, and its value as an additional prognostic tool. A limitation of scan-based technologies such as MRS (also MRI, CT, and PET) is that they give "snapshots" done usually just once or twice during each patient's neurocritical care, and the question arises of optimally integrating scan-based data with continuous bedside monitoring modalities (2). A detailed description of magnetic resonance (MR) physics is outside the scope of this review and can be found in the literature (15–17). However, we cover a simplified explanation of the relevant basic science of MRS and practical considerations of scanning patients with acute severe TBI.

Magnetic Resonance Spectroscopy

Certain nuclei possess a property termed "spin" that enables detection by MR. Examples include ^{1}H, ^{31}P, and ^{13}C (which all possess spin of 1/2). Nuclei with 0 spin, e.g., ^{12}C, cannot be detected by MR. For illustration, nuclei with spin can be considered as tiny, atomic, bar magnets. MR detection relies on the principle that when a population of magnetic nuclei is placed in an external magnetic field, the nuclei become aligned in a predictable number of orientations. For ^{1}H (likewise ^{13}C or ^{31}P), there are two orientations: with or against the external magnetic field. Since the with-field orientation is preferred as lower energy, slightly more of the population of nuclei are aligned with the field than against the field. Some spins align against the field, as the nuclei are very weak magnets and the energy difference between the two orientations—with and against the external field—is not large, even in a strong external magnetic field. There is enough thermal energy at physiological temperature for nuclei to exchange between the two orientations, though with a slight excess on average in the lower energy (aligned with field) state. MR spectroscopy measurement applies energy as radio-frequency (RF) electromagnetic radiation to excite the small excess of with-field oriented nuclei into the against-field higher energy state. When the RF is removed, the energized nuclei relax back to the lower energy with-field state, and in doing so the relaxing nuclei create their own fluctuating magnetic field. This induces a current in the receiver coil that is around the "sample" (e.g., brain). This current constitutes a signal that is electronically converted into a peak in the spectrum.

For the signal from a nucleus to be detected by *in vivo* MRS the molecule in which it is present must be sufficiently mobile and free to tumble. In the case of nuclei that are bound up in large macromolecules or closely confined by cellular membranes, the spins of the nuclei relax (by spin–spin interaction with other nuclei) too quickly for detection and characterization by *in vivo* MRS.

The RF needed to excite the nucleus depends on what isotope it is (e.g., ^{1}H, ^{31}P, or ^{13}C), its chemical environment and the

strength of the external magnetic field, i.e., the scanner magnet (18). The RF needed to excite the nucleus is directly proportional to both the strength of the external magnetic field and the gyromagnetic ratio (see **Table 1**) of the isotope. The effect of chemical environment is relatively much smaller, but readily measurable. It is due to greater or lesser shielding of the nucleus from the main (external) magnetic field by the electrons surrounding the nucleus. This electron shielding results in small changes of the frequency of the MR signal detected and is called the chemical shift, usually expressed as parts per million (ppm; Hz per MHz). It is the same at all field strengths and is the basis for metabolite identification using MRS. In principle, a peak will be observed for every magnetically distinct nucleus in a molecule because nuclei that are not in identical structural situations do not experience the same shielding, and therefore experience slight differences in external magnetic field.

Magnetic resonance spectroscopy spectra are typically plotted with chemical shift along the *x*-axis with increasing (positive) chemical shift values reading from right to left (**Figures 2** and **3**). The *y*-axis represents signal intensity. The size (height and area) and shape of a peak are dependent on the concentration of metabolite(s) that it represents, relaxation time (T1/T2) effects, and splitting by spin–spin coupling. The latter, termed J-coupling, which occurs most strongly between magnetic nuclei that are adjacent to each other causes splitting of their spectral peaks (some splitting by more distant nuclei can also occur). J-coupling can reveal further information about the structure of a nucleus's molecular environment, but in practice resolution is rarely sufficient with *in vivo* MRS to fully separate a multiplet and so the effect of peak splitting usually just broadens the signal and reduces peak height relative to baseline noise. Spectra can be simplified by ^{1}H decoupling, which may be necessary in some applications (see ^{13}C MRS), but is of limited value in others (see ^{31}P MRS).

As signal frequency differences are used for chemical shift metabolite identification and not for spatial encoding, alternative methods of localization must be used to exclude erroneous signal from non-neural tissue and acquire spectra from chosen regions of interest: a single voxel of brain can be selected using dedicated pulse sequences and gradient magnetic fields such as point-resolved spectroscopy (PRESS), or multivoxel chemical shift imaging (CSI) that uses phase encoding to sample spectra from multiple voxels at the same time (**Figures 2** and **3**) (18, 19). Outer volume suppression can also be used to suppress signal from scalp and bone (20), and where on a patient's head a surface coil is placed will affect the region of the brain that it samples. Surface coils (**Figure 4A**) are more sensitive than volume coils (**Figure 4B**) that envelope the head, but suffer from a less homogenous delivery of RF pulse to the brain. Due to the different frequencies of ^{1}H, ^{31}P, and ^{13}C, they each require dedicated RF coils that are tuned to their respective frequencies (see **Table 1**). ^{31}P and ^{13}C coils will typically contain an additional ^{1}H channel within their housing, however, for simple brain imaging to localize the spectra, and for decoupling.

Magnetic resonance scanners are generally classified by their magnetic field strength. Most clinical scanners are either 1.5 or 3 T, which are sufficient for standard MRS studies but higher fields such as 7 or 9.4 T exist. Higher field strength generally results in better spectral resolution and signal-to-noise ratio but comes with the trade-off of greater magnetic field inhomogeneity and RF power deposition into the body resulting in greater tissue heating (21, 22).

In vivo MRS studies often express metabolite concentrations as ratios of one another. Whereas the peak area of an MRS spectrum is proportional to the number of excited nuclei within the measurement volume, it is also affected by various other variables: the timing of pulse sequences and their interaction with relaxation times, magnetic field inhomogeneity, and particularly RF coil loading, which will vary between subjects and with coil position (23). To compensate for all these effects is technically very challenging, even with external phantoms, as the latter may not accurately mimic tissue properties, and some biochemicals may be unstable. Expressing metabolites as ratios removes the need for units and calibration although ratios can be more difficult to interpret than absolute concentrations. Application of an artificial reference pseudo-signal is an approach that shows promise for absolute quantification of concentrations in MRS (23).

Quantification of MRS signals, whether absolute or ratios, requires fitting of the spectral peaks. Simple integration to measure peak areas is not adequate in MRS as there is overlap between signals and the spectra are further complicated by noise. Therefore, MRS data are fitted using specialized algorithms that are available as various software packages, e.g., LCModel (24), jMRUI (25, 26), and Syngo on Siemens scanners (Siemens Healthcare GmbH, Erlangen, Germany).

TABLE 1 | Hydrogen, phosphorus, and carbon gyromagnetic ratio, Larmor frequency at 3 T, % natural abundance, and relative sensitivity to ^{1}H magnetic resonance spectroscopy accounting for % natural abundance of the isotopes and Larmor frequency, but not natural concentration of biomolecules in the brain.

Isotope	Gyromagnetic ratio (MHz T^{-1})	Larmor frequency at 3 T (MHz)	Natural abundance (%)	Relative sensitivity
^{1}H	42.58	127.74	99.99	1
^{31}P	17.24	51.72	100.00	0.0665
^{13}C	10.71	32.13	1.11	0.00018

Adapted from de Graaf (15).

^{1}H MRS

Hardware and Sensitivity

As MR imaging of the brain employs the detection of the ^{1}H nucleus in water standard clinical head coils can perform ^{1}H MRS. With its relatively high sensitivity, this has resulted in ^{1}H MRS being the most studied spectroscopy technique in the investigation and monitoring of TBI.

^{1}H can be found in most organic molecules, but for a metabolite to be detected by *in vivo* ^{1}H MRS it must be present at millimole per liter (mmol/L) concentrations and be freely mobile: not bound to or closely confined by membranes or macromolecules. If it is, the signal from its ^{1}H signal decays away too quickly and is either not detected or lost in the baseline (27). As the concentration of water in the brain is ≈50,000 mmol/L ^{1}H MRS requires

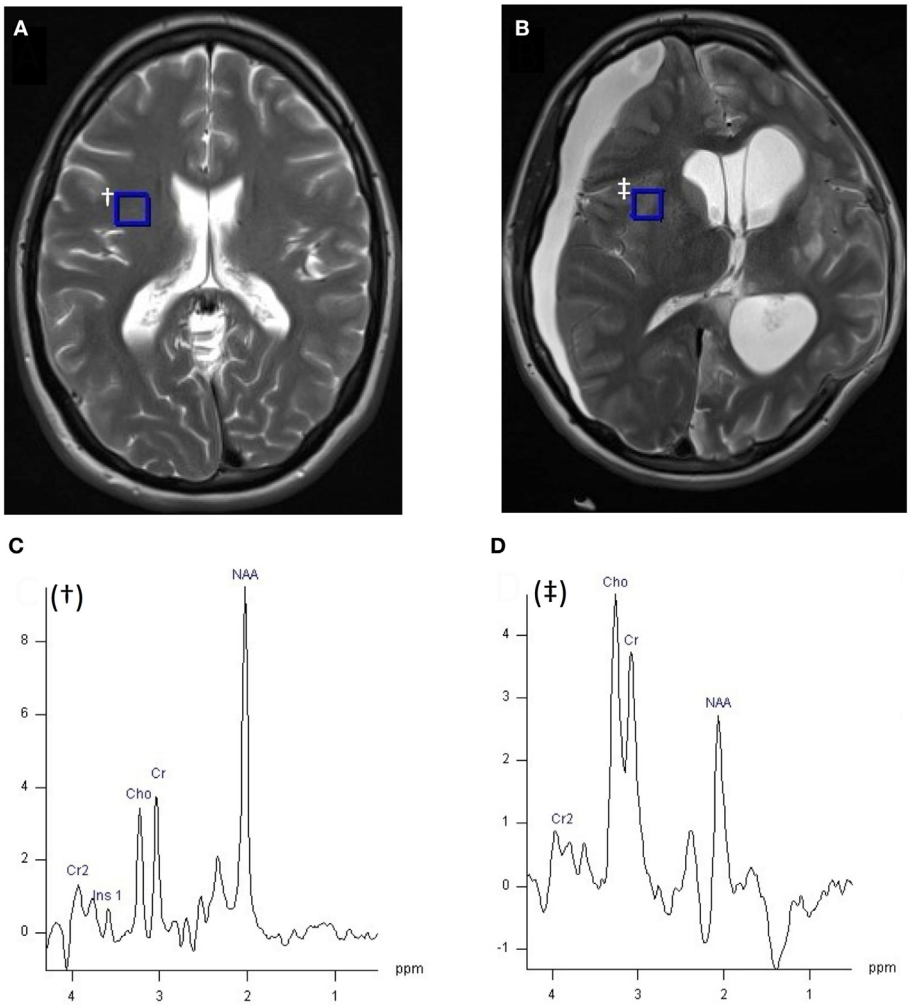

FIGURE 2 | ^{1}H MRI (T2W axial slice) and ^{1}H magnetic resonance spectroscopy chemical shift imaging (CSI) (echo time 135 ms, TR 2,200 ms, 3 averages, 4:41 min acquisition time, 200 ms Hanning filter) of healthy control **(A)**, and patient with acute severe traumatic brain injury after craniectomy **(B)** acquired with a 12 channel ^{1}H volume coil on a Siemens 3 T scanner, data analysis performed with Siemens Syngo software. Panel **(A)** demonstrates the position of the selected voxel (blue square, †), represented in panel **(C)**, within the CSI grid (hidden). **(C)** ^{1}H spectrum of 10 mm × 10 mm × 15 mm voxel † from healthy volunteer **(A)**. **(D)** ^{1}H spectrum of 8 mm × 8 mm × 15 mm blue square voxel ‡ of patient **(B)**, within the CSI grid (hidden). Metabolite peaks are annotated in panels **(C,D)**: Cr, creatine; Cho, choline; NAA, *N*-acetylaspartate, chemical shift on the *x*-axis in parts per million, signal intensity on *y*-axis using arbitrary units. Unpublished images by Tonny V. Veenith, courtesy of the Wolfson Brain Imaging Centre, Cambridge, UK.

water suppression to stop the huge water peak dominating the spectrum, masking the other metabolites of interest (28).

^{1}H MRS is typically performed with a long echo time (TE) of around 120–150 ms (29), which reveals a simplified spectrum of *N*-acetylaspartate (NAA), choline, creatine, and lactate (30). Using a very short TE of around 20–35 ms (28, 31, 32) allows detection of species whose magnetization, and therefore signal, decays more rapidly: Glu, Gln, myoinositol, and lipids. However, the gain in information comes with increased spectral complexity.

Creatine

The creatine singlet peak at 3.0 ppm in the ^{1}H spectrum represents both creatine and its phosphorylated form phosphocreatine (PCr). These are found in high concentrations in metabolically active tissues that require energy in bursts such as brain, muscle, and heart. PCr may rapidly donate its phosphate group to ADP, rapidly regenerating ATP by becoming creatine. In health, creatine is thought to vary less than other ^{1}H MRS metabolites throughout the brain so it is the most commonly used denominator when expressing ^{1}H metabolite ratios (33). PCr can also be detected by ^{31}P MRS (see ^{31}P MRS).

Effect of TBI

Despite creatine being often regarded as a stable brain metabolite, enzyme extraction studies of rat TBI have shown significant decline (up to 45%) in brain creatine hyperacutely following TBI (34). Conversely, in a human study of mild TBI, creatine was found to be elevated in the splenium of the corpus callosum and

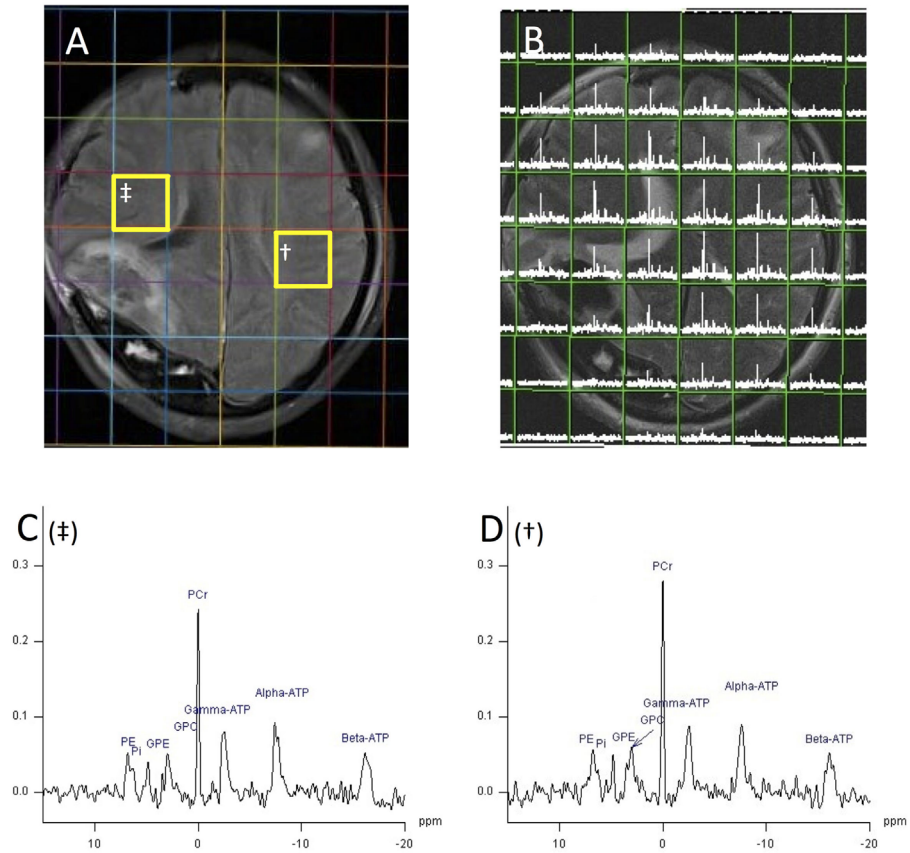

FIGURE 3 | ^{1}H magnetic resonance spectroscopy (MRI) and ^{31}P MRS chemical shift imaging (CSI) (echo time 2.30 ms, TR 4,000 ms, 25 mm voxels, 30 averages, 18 min acquisition time, 200 ms Hanning filter) of patient with acute severe traumatic brain injury acquired with a ^{31}P birdcage volume coil (PulseTeq, Chobham, Surrey, UK) on a Siemens 3 T scanner, data analysis performed with Siemens Syngo software. **(A)** Axial FLAIR image demonstrating decompressive craniectomy on patient's right side with associated regions of high signal in that hemisphere. **(B)** Axial T2 HASTE acquired with ^{1}H channel on a ^{31}P coil overlaid with ^{31}P MRS CSI grid of 8 × 8, 25 mm cubed voxels. Each voxel contains the spectrum from its volume. **(C,D)** ^{31}P spectrum from voxel † **(D)** and ‡ **(C)** of image 5A with phosphorus peaks annotated. Species can be identified by their chemical shift on the *x*-axis in parts per million. PE, phosphomonoesters; Pi, inorganic phosphate; GPE, glycerol 3-phosphorylethanolamine; GPC, glycerol 3-phosphorylcholine; PCr, phosphocreatine; ATP, adenosine triphosphate. Signal intensity on *y*-axis using arbitrary units. Unpublished images by the authors, courtesy of the Wolfson Brain Imaging Centre, Cambridge, UK.

white matter of the cingulate gyrus compared to healthy controls, thought to be due to higher energy demand after TBI (35). Many studies do not report a change in creatine after TBI hence creatine is often used as an internal reference for measurement of other metabolites, but these examples demonstrate that the possibility of changes in creatine concentration cannot be ruled out when relying on it as a reference ratio.

N-Acetylaspartate

The NAA peak at 2.0 ppm is a singlet that represents NAA and its product *N*-acetylaspartylglutamate, whose small peak is not resolved from the main NAA peak. NAA is formed from aspartate and acetyl-CoA by L-aspartate *N*-acetyltransferase which are associated with endoplasmic reticulum, or by splitting of *N*-acetylaspartylglutamate by *N*-acetylated-a-linked-amino dipeptidase (27, 29). Its specific role is not fully understood but it is closely associated with mitochondria and ATP (36). NAA is found predominantly in neurons and is thought to be a marker of neuron viability where it is transported down their axons, released, and taken up by oligodendrocytes where it is broken down into acetate and aspartate (35, 37). The role of NAA in myelin lipid synthesis, particularly in early development, is well established. The acetic acid from NAA becomes incorporated into CNS myelin (38). Under metabolic stress, a shortage of acetyl-CoA could result in reduced NAA synthesis and increased hydrolysis of NAA to provide acetate for myelin repair (39, 40). Among other functions ascribed to NAA is the idea that it is involved in osmoregulation (38). Normally, NAA/Cho ratios are higher in gray matter than white matter (29) and can be low due to any cause of neuronal loss. NAA concentrations can be up to 7.5–17 mmol/L in brain; equal to that of the main excitatory neurotransmitter Glu (15, 35).

Early Changes after TBI

Studies of hyperacute changes to brain metabolism following TBI are generally limited to experimental animal models due to the time delay transferring patients to hospital and stabilizing them before MRS can be performed. Animal studies show a rapid fall

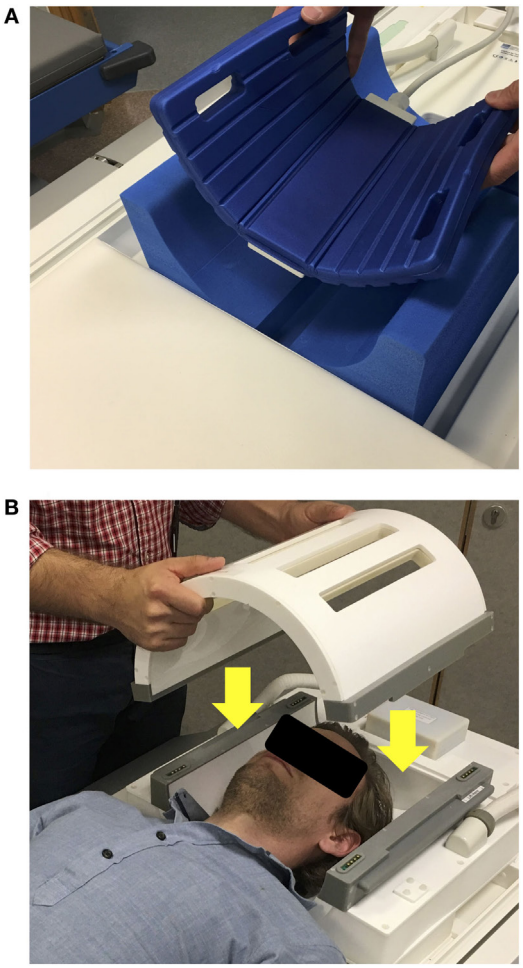

FIGURE 4 | **(A)** Example of a ^{13}C surface coil (Rapid Biomedical GmBH, Rimpar, Germany) with flexible design, allowing it to come in closer contact to the patients' head. Here, it is positioned to sample the occipital lobe. The coil contains a ^{13}C channel and ^{1}H channel within its housing. **(B)** Example of a ^{31}P birdcage volume coil (PulseTeq Ltd., Chobham, Surrey, UK), which can be opened, allowing to access a patient's head. The coil also contains a ^{1}H channel for imaging to allow spectral localization.

of NAA within the first hours following TBI, proportionally to the severity of the insult that can reach its lowest level at 48 h after injury (41–43). This initial rapid decline in NAA following TBI likely represents a disruption in neuronal NAA production through general micro-architectural disruption and mitochondrial dysfunction (35, 42). Human studies of patients with acute severe TBI performed within 24 h also show a reduction in NAA, NAA/creatine, and NAA/choline compared to healthy controls (44–47). Another study of 10 patients with moderate–severe TBI studied slightly later, after 48–72 h after injury also found a reduction in NAA in ^{1}H MRS compared to healthy volunteers, and the reduction was correlated with injury severity (GCS at presentation) (48).

^{1}H MRS performed in the subacute period around 1 week following acute severe TBI typically demonstrate persisting lower NAA/creatine ratios than healthy controls (29, 49, 50), which continued to fall in one study (29). Interrogation of peri-lesional brain typically showed even greater NAA decline through the subacute period, beyond 10 days (29).

Later Changes after TBI

If the primary injury is not too severe or compounded by further metabolic stress such as hypoxia or hypoperfusion, mitochondrial function and NAA may recover over the preceding days and months (41) with preservation of the neuron population. If the injury is more severe, there is likely irreversible physical and metabolic damage to the neurons which leads to a significant decline in neuronal population and therefore no recovery of NAA on MRS studies.

Studies of delayed ^{1}H MRS performed in the chronic, recovery phase after acute TBI either show recovery of NAA back to the levels seen in healthy controls in patients who make a good recovery or a persisting depression of NAA measured by ^{1}H MRS in patients with poor long-term neurological outcome (29, 51). An exception to this is regions of brain surrounding significant traumatic lesions which tend not to recover despite patients having a good recovery (29), and a study by Garnett et al. who found persisting NAA depression in all patients, regardless of outcome (49). Contrastingly in other pathologies, partial recovery of brain NAA levels was reported using ^{1}H MRS in a small follow-up study of acute brain damage (non-TBI) patients (52).

Chronic NAA depression may affect white matter more than gray matter following severe TBI, as studies of patients at 6 weeks to 6 months after TBI found reduced NAA in the white matter and not gray matter (51, 53). This may also be explained by most studies selecting regions of the brain predominantly represent white matter and the corpus callosum.

Role in Clinical Care

Measuring NAA using ^{1}H MRS can be clinically valuable due to its correlation with patient prognosis: the severity of depression of NAA/total metabolites (48), NAA/Cho (29), and NAA/Cr (49, 54) measured in the acute and subacute phase of injury correlates with patient outcome. Whereas these studies predominantly selected subcortical white matter and corpus callosum, the recovery of NAA in the thalami of TBI patients acutely after injury has been shown to predict good outcome (55). Another study of brainstem ^{1}H MRS in 40 patients with severe TBI showed that at a median 17 days after injury NAA/Cr ratio could predict very poor outcome in some patients that did not have visible injury on MRI. Furthermore, when included in a principal component analysis with FLAIR and T2* imaging, MRS allowed accurate prediction of GOS I–II, GOS III, and GOS IV–V outcomes when these modalities alone could not (56).

Choline

The choline peak at 3.2 ppm is formed from free choline, phosphocholine, and glycerophosphocholine (15). Choline is a precursor of acetylcholine; an important neurotransmitter that is also found at high concentrations bound to cell membrane phospholipids. In its bound form its T2 is too short for detection, but when it is liberated during cell membrane turnover or cellular production

of acetylcholine it becomes visible. An increase in choline is used to identify increases in cell membrane turnover or destruction in aggressive brain tumors and demyelinating disease, but in normal brain it is found at 0.5–2.5 mmol/L (15).

Early Changes after TBI

Following TBI, a raised choline is thought to represent cellular damage through membrane breakdown. Elevated choline/creatine compared to healthy controls has been found both subacutely after injury and in the chronic phase (49, 57). Garnett et al. found choline/creatine increased in proportion to the severity of injury in normal-appearing white matter (49), but Wild et al. found no such correlation (57), although this could be due to changes in creatine blunting the effect of any relative change. An elevation of choline/total metabolites has been demonstrated within 48–72 h of moderate–severe TBI, but this also did not correlate with presentation GCS or outcome at 3 months (48).

Later Changes after TBI

^{1}H MRS performed in the subacute period following moderate-minor TBI of 40 patients found elevated choline/NAA ratio throughout the cerebrum and cerebellum (56). However, there was an inverse relationship with outcome as patients with higher choline/NAA ratios had better cognitive performance at recovery. Delayed choline measurement during the chronic phase of severe TBI recovery often demonstrate persisting elevated choline/creatine and reduced NAA/choline (49, 51) that sometimes correlates with functional status at the time (58).

Role in Clinical Care

Choline can potentially be used as a predictor for TBI prognosis. A study of 42 patients with subacute (7 days post-injury) severe TBI found that choline elevation in occipital gray and parietal white matter predicted outcome with 94% accuracy (32). However, a separate smaller study (10 patients) performed in the acute period (48–72 h) did not find a correlation with degree of choline elevation and outcome (48). It is not clear why the magnitude of the acute choline rise does not correlate with the severity of the initial injury or later functional outcome of the patient. Delayed choline measurements tend to be more closely associated with outcome (32, 49) which may be because choline represents active neuroinflammation causing further cell membrane disruption and injury, well after the initial TBI (59, 60). If this is the case, ^{1}H MRS could be used to identify patients at risk of neuroinflammation; selecting them for potential new anti-neuroinflammatory therapeutic agents (61).

Myoinositol

Myoinositol is a precursor of both phosphatidylinositol and phosphatidylinositol 4,5-bisphosphate. Its ^{1}H MRS peak is at 3.56 ppm and normal concentration in the brain is 4.0–9.0 mmol/L. It is regarded as a cerebral osmolyte and astrocyte marker. Variable changes are seen in different intracranial pathologies: an absolute decrease may be seen in stroke and hepatic encephalopathy, likely due to imbalance of osmoregulation, while an increase in myoinositol is found in astrogliosis, although when this is expressed as a ratio of myoinositol/creatine the effect disappears (62).

Effect of TBI

Pascual et al. showed that myoinositol can increase in the first 24–48 h after TBI in a rat model (63). A study of 38 pediatric TBI patients showed occipital gray matter myoinositol levels were increased in children with TBI compared to healthy controls and that higher myoinositol levels correlated with poor outcome (64).

Glu and Gln

Glutamate and Gln are amino acids found in abundance in the human brain detected at 2.2–2.4 ppm in a ^{1}H MRS spectrum. Glu is the main excitatory neurotransmitter in the brain and is stored in neuron vesicles, found at a concentration between 6.0 and 12.5 mmol/L in healthy human brain (15). After release, it is taken up by glia and converted to Gln, which is then fed back to neurons in the Glu–Gln cycle (65). Gln is found in the brain at concentrations of 3.0–6.0 mmol/L (15). The molecular structure of Glu and Gln is sufficiently similar that it is difficult to distinguish between their chemical shifts [2.04–2.35 and 2.12–2.46 ppm (15)] on an *in vivo* ^{1}H MRS examination. Thus, the term "Glx" is used to represent the combined pool of both metabolites.

Effect of TBI

During TBI, there may be intensive neuronal activation associated with impaired Glu reuptake and transport that causes Glu associated excitotoxicity (32, 66, 67). Shutter et al. found combined Glu and glutamine (Glx) were significantly elevated in occipital gray and parietal white matter early after injury (7 days) in patients with poor outcome at 6 and 12 months after TBI and combined Glx and Cho ratios predicted long-term outcome with 94% accuracy when GCS motor score was included in the model (32).

Gamma-Aminobutyric Acid (GABA)

Gamma-aminobutyric acid is the main inhibitory neurotransmitter of the brain and, like Glu, is stored intracellularly in neuron vesicles at concentrations of up to 1 mmol/L in the brain (68). After release, it is taken up by glia and converted to Gln *via* Glu and fed back to neurons. Its ^{1}H MRS peak is found between 2.2 and 2.4 ppm which overlaps with the Glx species and thus is very difficult to quantify (68, 69). GABA plays a role in epilepsy and can be increased in patients with epilepsy by treatment with common anticonvulsants. However, other studies have shown no difference between patients suffering with epilepsy and normal healthy controls (68). GABA quantification can be improved by acquiring the spectra using specialized GABA-editing techniques such as the pulse sequence MEGA-PRESS (70, 71).

Effect of TBI

Gamma-aminobutyric acid normally modulates the excitatory pathways in the brain. Following TBI a loss of GABAergic neurons disrupts the balance of excitation and inhibition, leading to further cell injury and apoptosis (72). An imbalance of GABA and Glu after TBI may also result in post-traumatic epilepsy but measurements of GABA are rarely reported in human ^{1}H MRS studies and GABA has only been shown to fall after TBI by 46% within 24 h in a single animal study.

Lactate

Most of the lactate in the brain is regarded as "glycolytic," originating from glucose metabolism *via* the Embden–Meyerhof pathway, to pyruvate, followed by conversion of pyruvate to lactate by the action of LDH. There is some disparity in nomenclature about glycolysis in the brain literature, which undoubtedly adds confusion, as glycolysis culminating in lactate is often termed "anaerobic metabolism," though often without supporting evidence regarding the oxygen status in the tissue concerned. In old studies brain injury was often associated with hypoxia/ischemia (real or assumed), although modern neurocritical care means that overt hypoxia/ischemia is usually avoided. Even so, microvascular ischemia appears to exist in some cases (73), as do episodes of hypoxia (74). We regard hypoxia as $PbtO_2$ <20 mmHg, with severe hypoxia as $PbtO_2$ <10 mmHg.

The ability of lactate to act as a neuronal fuel has now also been established (6, 75) although its importance relative to glucose is debated (76). Lactate may be elevated by hypoxia, ischemia, or macrophage infiltration (77). It can appear as a characteristic doublet at 1.3 ppm when acquired with a long TE (TE 144 ms), but the MR behavior of lactate is complex, and lactate signals can virtually disappear or appear inverted depending on MR conditions (78). Interpretation of lactate is further complicated by overlap with lipid signals. Lactate is typically represented by a small peak on ^{1}H MRS despite its relatively high extracellular concentration of 2.9 mmol/L (79) as its concentration intracellularly [which dominates brain volume (13, 14)] is much lower.

Effect of TBI

In TBI, the elevation of brain extracellular lactate is known to be associated with poor prognosis. Although lactate is a normal component of energy metabolism, if lactate appears elevated in a tissue on ^{1}H MRS it is usually a sign of pathology. Lactate elevation does not necessarily indicate hypoxia, as the phenomenon of "aerobic glycolysis" whereby cells produce lactate despite a seemingly adequate supply of oxygen is well known, e.g., the Warburg effect in tumors, and a similar effect is seen in TBI, where it is variously termed metabolic dysfunction, mitochondrial dysfunction, and, in extreme cases, metabolic crisis. In early work on rat models of TBI there appeared to be an initial rise in brain lactate hyperacutely following moderate or severe injury, associated with persisting neurological dysfunction at 4 weeks (80, 81). Lactate returned to normal after about 60 min, and there was no association between magnitude of hyperacute transient lactate rise, injury severity or neurological outcome. However, mild injury that did not result in long-term neurological deficit did not cause any increase in lactate (80). The hyperacute period is only addressable in experimental models, and study is not feasible in human TBI patients, as typically an hour or more will have elapsed before they arrive at hospital and longer until a scan can be performed. In human TBI, lactate elevation can be seen on ^{1}H MRS in some but not all instances, illustrated in Marino et al. (48). Because of the complications with lactate signals (see above) some ^{1}H MRS studies of normal and TBI brain do not consider lactate at all (82). Lactate elevation is most markedly seen in pediatric head injury (83, 84). Makoroff et al. showed that in four pediatric TBI patients elevation of lactate measured by MRS was due to hypoxic–ischemic injury, which was associated with worse early neurological outcome scores (85). In adult TBI patients, lactate (measured by MRS) is similarly only raised if there is a severe ischemic process where it may rise diffusely in the brain within 48–72 h of injury (48). This rise can persist for weeks (86), and the degree of lactate elevation may correlate with outcome at 3 months; higher lactate corresponding to worse outcome (48).

Lipid

Lipids and phospholipids form a group of peaks at 1.3 ppm. When lipid is bound in intact cell membranes its T2 is too short for detection by *in vivo* ^{1}H MRS. Elevated lipid suggests significant cell membrane disruption so is only visible in severe trauma, such as in shaken baby syndrome (87). Lipid measurements are not often reported in adult TBI studies.

Summary of ^{1}H MRS in TBI

Following TBI, the brain may suffer from significant metabolic failure, direct cell damage, hypoxia, and neuroinflammation. These can be detected non-invasively using ^{1}H MRS, prompting intervention: metabolic failure signified by NAA reduction may allow a patient's metabolic support to be altered by administering an infusion of glucose, or newly developing metabolic treatments for mitochondrial failure such as succinate (88).

Prognosticating in acute severe TBI is challenging. Several metabolites, including NAA, choline, myoinositol, Glx, lactate, and lipid may help predict patients who will not survive or are likely to survive with the most extreme disability (32, 48, 49, 54–56). ^{1}H MRS can help clinicians and patients' families in terms of prognosis. As acute severe TBI typically results in both a fall in NAA and a rise in Cho that are associated with outcome, the NAA/Cho may be the most appropriate indicator of injury, distinguishing patients with good and poor outcome (32). This has the potential to reduce patient and family suffering and conserve intensive care resources.

The most appropriate region of the brain to be interrogated for prognostication is unclear. CSI measurements of the subcortical white matter with inclusion of the corpus callosum would be the most comparable to the literature (29, 48, 49, 54), and the inclusion of single voxel brainstem NAA measurement would allow MRI invisible injury to this critical structure to be detected (56). Other potential targets are the occipital and parietal lobes where changes in Glx, myoinositol, and Cho have been correlated with patient outcome.

A summary of the effect of TBI on metabolites interrogated by ^{1}H MRS are shown in **Table 2**.

^{31}P MRS

In vivo ^{31}P MRS detects unbound molecules that contain phosphorus in the human brain. The most notable of these are the fundamental molecules of chemical energy in all eukaryotic organisms: ATP, ADP, adenosine monophosphate (AMP), PCr, and Pi. As well as providing information about energy status, Pi

TABLE 2 | Summary of metabolite changes following traumatic brain injury (TBI) detectable with *in vivo* ^{1}H magnetic resonance spectroscopy.

	Spectrum peak (ppm)	Physiology	Change in acute TBI	Change in chronic TBI	Correlation to prognosis
N-acetylaspartate	2.02	Neuron viability	↓↓	↓	✓
Creatine	3.02	Adenosine triphosphate generation and energy metabolism	↔		
Choline	3.24	Cell membrane turnover	↑↑	↑	✓
Myoinositol	3.5	Osmoregulation	↑		✓
Glx [glutamate (Glu) + glutamine]	2.2–2.4	Excitatory neurotransmitter (Glu)	↑↑		✓
Lactate	1.33	Mitochondrial dysfunction	↑		✓
Gamma-aminobutyric acid	2.2–2.4	Inhibitory neurotransmitter	↓		
Lipid	1.3	Cell membrane	↑		✓

↑: increase in metabolite; ↓: decrease in metabolite; ↔: no significant change or insufficient data; ✓: potential clinical use as a prognostic predictor.

allows measurement of brain pH through changes in its chemical shift (89–91). Phosphomonoesters (PMEs) and phosphodiesters (PDEs) are also metabolites that contribute to a standard ^{31}P brain spectrum and are thought to represent cell membrane turnover.

Hardware and Resolution

Magnetic resonance spectroscopy detection of ^{31}P is less sensitive than ^{1}H. Comparing the two isotopes, for the same number of nuclei in the same external magnetic field, the relative sensitivity, also termed receptivity, is calculated from the NMR sensitivity [proportional to $|\gamma^3| \times I\ (I + 1)$] multiplied by the natural abundance (15). Since I (the spin) is 1/2 for both ^{31}P and ^{1}H, and the natural abundance is over 99.9% for ^{1}H and 100% for ^{31}P, the gyromagnetic ratio γ is the crucial factor: 26.752 and 10.831 (units 10^7 rad T^{-1} s^{-1}) so relative sensitivity (vs. ^{1}H) is only 0.065 for ^{31}P, so just 6.5% (15). In layman's terms, the gyromagnetic ratio γ, can be thought of as the strength of the tiny magnets that are the ^{31}P and ^{1}H nuclei, divided by their spin (value 1/2 here in both cases)—thus ^{31}P is less sensitively detected than ^{1}H, because the ^{31}P nuclei are weaker magnets than ^{1}H nuclei.

To acquire phosphorus spectra with acceptable signal to noise, either larger voxels must be selected compared with ^{1}H MRS and/or more averages acquired, resulting in longer scan times. ^{31}P MRS is also limited by the pulse sequences for localization that can be used: ^{31}P metabolites have relatively short relaxation times so the transverse magnetization must be sampled as quickly as possible after excitation (short TE). Single volume spectroscopy techniques PRESS and STEAM use multiple echo steps that require long TE, so ^{31}P MRS localization is limited to single voxel ISIS and multivoxel CSI in the brain (92).

The range of chemical shifts that the main metabolites in an *in vivo* ^{31}P MRS spectra occupy is also much wider (≈30 ppm) than that of ^{1}H MRS (≈5 ppm). The chemical shifts of PCr and Pi are dependent on pH, and α-ATP and β-ATP on the concentration of free magnesium (Mg^{2+}). PCr is conventionally considered a reference at 0 ppm (by definition), and the chemical shifts quoted below represent those from its center at a pH of 7.2 with normal tissue Mg^{2+}, as per de Graaf (15).

High-Energy Phosphates

The high-energy phosphates detected by ^{31}P MRS (PCr, ATP, ADP, AMP, and Pi) are directly related to each other chemically: the high-energy phosphate group passes from pool to pool reaching a state of equilibrium depending on the energy expenditure and generation within the cells. This contrasts with metabolites studied by ^{1}H MRS which are linked to each other in a broader, biological sense. Thus, we will consider the high-energy phosphates as a group, with relative ratios of interconnected metabolites; rather than as individual species.

ATP Hydrolysis and Generation

Adenosine triphosphate is the fundamental molecule of chemical energy in eukaryotic and prokaryotic organisms and is used and then regenerated with rapid turnover in the brain (93). The hydrolysis of ATP into ADP + Pi releases energy that is harnessed to drive the main cellular processes including the sodium potassium exchanger (Na^+/K^+ ATP_{ase} pump) that maintains the membrane potential in neurons. The brain maintains ATP at a concentration several fold higher than that of ADP [average 3 vs. 0.1 mmol/L (15, 94, 95)] to drive these processes by continually recycling ADP back to ATP. This is done through glycolysis, the citric acid cycle and the electron transport chain in mitochondria where the enzyme ATP synthase catalyzes the conversion of ADP and Pi to ATP down a hydrogen ion gradient, provided oxygen is available as a terminal electron acceptor on complex IV. This cycle occurs continually so that the human brain, weighing about 1.2 kg, uses an estimated 5.7 kg of ATP per day (93).

Creatine Kinase

The process of ATP regeneration *via* ATP synthase is relatively slow on a cellular scale so tissues that require energy in bursts such as the brain, skeletal muscle, and cardiac muscle contain creatine and PCr. Catalyzed by the enzyme creatine kinase, PCr can very rapidly donate its high-energy phosphate group to ADP, rapidly regenerating ATP during periods of high metabolic demand independently of oxygen. During periods of lower metabolic demand, the PCr store is replenished in the mitochondrial intermembrane space, again by creatine kinase from newly generated ATP. PCr is a spatial buffer for ATP as well as a temporal buffer. Most ATP is produced in the mitochondria but used in the cytoplasm. The free diffusion distance of ATP and ADP is limited by their strong negative charges and low cellular concentrations whereas PCr and Cr diffuse more freely due to their smaller size, less overall charge, and higher concentrations. The PCr–Cr system therefore acts as a shuttle linking ATP production in the mitochondria to its use in the cytoplasm (96–99).

^{31}P Peaks and Their Metabolites

The PCr signal, whose chemical shift is defined by convention as 0.00 ppm, is the most easily identifiable peak in brain ^{31}P MRS. Brain PCr concentration has been reported at 4.0–5.5 mmol/L (15) concentrations at reasonably constant levels between gray and white matter (15, 100, 101).

The β-ATP peak represents phosphorus in the middle phosphate group; a structure that is unique to ATP (15). It would appear to be the most appropriate peak to represent ATP concentration, but its location at extreme upfield (−16.26 ppm) can make it difficult to excite consistently with a homogenous RF pulse that also covers the other metabolites.

γ-ATP is often used to represent the concentration of ATP, instead of β-ATP. At −2.48 ppm γ-ATP represents the distal phosphate groups of both ATP and ADP, which are effectively indistinguishable from each other *in vivo* due to their similar immediate chemical and nuclear environment. However, ADP is found at much lower concentrations in the brain (0.1 mmol/L) than ATP (3 mmol/L) (15, 94, 101), and ADP is regarded as mostly bound up in vesicles and mitochondria so poorly responsive on MR, making its contribution to the γ-ATP peak negligible.

α-ATP at −7.52 ppm represents the proximal phosphate groups in ATP and ADP and the central phosphates of NAD and NADH; these are poorly resolved in most *in vivo* MR spectra. The inclusion of NAD and NADH and its profile slightly further from the center of a typical RF pulse makes it an inferior choice to the γ-ATP peak for ATP characterization (15).

Inorganic phosphate is found at 5.02 ppm as a relatively small peak. Its small size can make it difficult to accurately integrate, but nevertheless it is often used to express ratios of brain energy ^{31}P species (102–104). Pi is a useful indicator of intracellular pH, which can be calculated from the difference in chemical shift between PCr and Pi (90, 105). Although Pi is may be a small peak some studies have shown existence of two Pi signals; ascribed to two pools of Pi differing in pH (ΔpH ~0.4) (106). In brain, the major (upfield) peak is assigned as intracellular Pi, and the minor (downfield) peak extracellular Pi, and the two signals have different T1 relaxation times, presumably reflecting the different environments surrounding the phosphate molecules.

Changes after TBI

PCr/ATP and PCr/Pi are two of the most commonly used ratios to express brain energy status. If the brain is metabolizing normally there will be sufficient ATP and plenty of its short-term high-energy store, PCr. However, if the brain is stressed, a plausible scenario is that it might draw on its store of PCr to maintain ATP homeostasis leading to a reduction in the PCr/ATP ratio and PCr/Pi ratio. The PCr/Pi ratio will also be affected by a potential increase in free Pi as ATP is hydrolyzed but not remade sufficiently in the mitochondria. PCr/Pi can be inaccurate with difficulty in reliably measuring the small Pi peak in a potentially noisy baseline.

Hyperacute ^{31}P MRS studies of TBI are limited to animals for the same reason as ^{1}H MRS. Ishige et al.'s study (103) of focal TBI in rats with sequential measurements after injury demonstrated a rapid fall in absolute PCr and an increase in absolute Pi in the first 15 min after injury. In the absence of further injury, these species recovered to near normal within 90 min (103). Further studies by Vink et al. of different grades of injury have demonstrated the same initial fall in absolute PCr and rise in absolute Pi (or fall in PCr/Pi ratio), which then recovers within ~100 min following moderate–severe trauma. There appears to be a second rise in PCr and fall in Pi and PCr/Pi ratio that occurs 120 min after injury, remaining depressed in severe injury (104, 107, 108). The initial falls in PCr/Pi were associated with brain acidosis in these studies, but the second falls were not. The degree of this second PCr/Pi depression 4 h after injury correlated with severity of insult, which itself correlated with 24 h neurological dysfunction (107). No studies demonstrated a decrease in ATP after moderate–severe injury. In the studies that included the most extreme injury severity, a different pattern was observed: a much greater, persistent fall in PCr and rise in Pi occurred that did not recover (108). Unlike animals subjected to more moderate grades of injury these animals also experienced an irreversible loss of ATP over the 3 h following injury (107, 108).

The addition of a secondary insult, hypotension, to experimental TBI greatly exacerbated the metabolic derangement measured by ^{31}P MRS. With moderate hypotension after TBI, a much greater immediate fall was seen in PCr which did not recover as well. Pi increased significantly more and the immediate acidosis was greater and did not recover as it did in the absence of hypotension. Importantly, ATP fell significantly in the presence of moderate–severe TBI with hypotension but not with TBI alone. Cells work very hard to maintain ATP homeostasis at the cost of other metabolites, so it appears that a fall only occurs in metabolic extremis following very severe injury, or when TBI occurs with additional hypotensive insult (103, 107–109).

An *in vivo* ^{31}P MRS patient study by Garnett et al. (102) of high-energy phosphates in the subacute period following TBI had different findings to those of the hyperacute TBI animal studies above. Seven patients with moderate and severe TBI were studied 9 days (mean) after injury: four patients had partially recovered and were self-ventilating whereas three were still intubated and ventilated. In normal-appearing white matter, a significant increase in PCr/Pi was found in patients with TBI compared to healthy controls, as was PCr/ATP (although non-significantly). The authors suggested that a possible explanation could be a change in cell population through reactive gliosis.

These studies suggest that ^{31}P MRS is detecting different changes in brain metabolism dependent on when after the injury the scan is performed. The initial fall in PCr seen in hyperacute animal studies (see above) is compatible with the interpretation of cell membrane injury causing K^+ efflux from cells, which leads to high demand on the Na^+/K^+ ATPase pump, consuming PCr. This initial fall in PCr recovered in these animal studies, but the second fall in the acute stage after 2 h did not and is of uncertain etiology. Cellular ATP appears to be maintained following all but the most severe forms of experimental TBI in animals, likely representing catastrophic energy failure with extreme, irreversible derangement of all phosphorus metabolites (103, 107, 108).

Brain pH and Mg^{2+} Concentration

The pH of the brain can be measured from the difference in chemical shift between the Pi and PCr peaks (89–91). Although

the small size of the Pi peak relative to baseline noise can lead to errors measuring its area, its chemical shift can generally be accurately identified. Changes in the concentration of hydrogen ions (pH) result in greater or lesser binding of H^+ ions to Pi. The presence of the additional hydrogen ions changes the proportion of protonated to un-protonated Pi which changes the mean chemical shift of the species population. Similarly, the concentration of brain Mg^{2+} can be calculated from the difference in chemical shifts between the α-ATP and β-ATP peaks (15, 89, 103, 104, 110).

Control of Brain pH

Normal neuronal activity causes constant changes in intracellular and extracellular pH in the brain which are buffered by several mechanisms: the PCr, ATP, and creatine kinase system is one of these. When creatine kinase catalyzes the regeneration of ATP from ADP and PCr, a H^+ ion is consumed: ADP + PCr + $H^+ \leftrightarrows$ ATP + Cr. Creatine kinase is strongly pH dependent and acts as both an ATP and pH buffer in cells with high metabolic workloads.

Effect of TBI

Rodent studies of hyperacute changes in brain pH following severe TBI have found an immediate, transient fall in pH for the first 15–60 min following moderate to severe TBI that is exacerbated by hypotension (80, 103, 104, 111). The magnitude of this transient acidosis does not correlate with neurological outcome, histopathological injury or severity of insult (80) for all but the most extreme (un-survivable) injuries where a progressive, terminal brain acidosis occurs (108). Changes in pH accompany changes in PCr/Pi ratio, returning to normal after an hour and a half in the absence of hypotension. This is what would be expected from the creatine kinase system, but it is not clear if a fall in PCr causes a shift of the equilibrium, and a rise in H^+ ions, or acidification causes a shift in the CK equilibrium and a fall in PCr. It is perhaps more likely that primary pH changes drive the PCr/Pi change as the delayed fall in PCr/Pi does not cause a change in pH, suggesting another mechanism.

Intracellular free Mg^{2+}, an important cofactor for glycolysis and oxidative phosphorylation, has been shown to fall by as much as 60–69% following animal experimental TBI (110–112), reaching its nadir between 1 and 4 h after injury. Free Mg^{2+} appears to be particularly sensitive to injury; declining significantly following moderate and even mild experimental TBI in the absence of changes to PCr, ATP, Pi and pH detected by ^{31}P MRS (43, 108, 110–112). Interestingly, in a graded TBI study performed by Vink et al. free intracellular Mg^{2+} did not fall in rats subjected to the most severe TBI. This was attributed to release of Mg^{2+} from the declining ATP that occurred in this group, replenishing the total level. After moderate injury Mg^{2+} appears to recover to baseline after about a week (43), but its calculation should be performed cautiously when spectra have low signal to noise as previous reported changes have been shown to be due to errors of chemical shift assignments (89). The subacute study by Garnett et al. of patients with moderate to severe TBI found white matter was more alkaline (higher intracellular pH) and had higher free intracellular Mg^{2+} in TBI patients 2–21 days (mean 9 days) after injury compared to healthy volunteers (102). A difference in gray matter pH was not found, although gray matter PCr/ATP was significantly higher in TBI brain than in healthy controls (102). Conversely, measurements of brain *extracellular* pH (not using MRS, but using intracranial probes) following severe TBI in humans suggest that lower pH is associated with a worse outcome (113, 114). The relationship between brain extracellular and intracellular pH in human TBI is unclear.

PMEs and PDEs

The cell membrane phospholipid bilayer in the brain is not visible on ^{31}P MRS because its magnetization decays too quickly for detection. Its precursors the PMEs phosphorylethanolamine (PE) and phosphorylcholine, are visible at 6.78 and 5.88 ppm in high quality spectra (15, 102). PDEs glycerol 3-phosphorylethanolamine and glycerol 3-phosphorylcholine, at 3.2 and 2.8 ppm, are produced by phospholipase breakdown of cell membranes. They are then converted to PMEs by phosphodiesterase. Consequently, the ratio of PME/PDE is thought to be an indicator of cell membrane turnover (92, 95, 115).

Changes in PME/PDE ratios are often explored in ^{31}P MRS TBI studies, but the small size of the PME and PDE peaks relative to baseline noise means that statistically significant differences often cannot be found, even if present (102). However, it should be noted that the phosphorus nuclei in PMEs and PDEs are coupled to hydrogen atoms, which causes splitting of their resonances that can be exploited with the polarization transfer technique and proton decoupling to significantly enhance their detection (15).

Confounders of ^{31}P MRS Measurements in the Brain

Regional variations of high-energy phosphate species in the human brain exist that influence the results obtained by ^{31}P MRS studies. Whereas the concentration of PCr remains relatively constant throughout the brain, PCr/ATP is higher in gray matter (GM = 1.19) than white matter (WM = 0.84) (116) because of the higher concentration of ATP found in white matter (GM = 2 mmol/L; WM = 3.5 mmol/L) (15). GM also has a higher metabolic rate than WM, using three times as much ATP and consuming 77% of total energy expenditure of the brain despite representing only 55% by tissue weight (116).

Phosphocreatine is known to vary with age in healthy volunteers: increasing age is associated with slightly higher PCr, lower PME and a slightly more acidic brain (117). There is also an inverse relationship between body mass index and absolute measures of PCr and ATP, but as these changes are equivalent there is no resulting change in PCr/ATP ratio (118).

If patients with acute severe TBI are studied whilst intubated, sedated and ventilated the effect of anesthetic agents should also be considered. There is evidence that phenobarbital increases the PCr of rat brain but does not change ATP or ADP, measured by biochemical assays on tissue extracts (119). Halothane, nitrous oxide and fentanyl do not seem to have any effect on high-energy phosphates concentrations (119).

Magnetization Transfer (MT) Technique

As well as measuring static concentrations of phosphorus metabolites (absolute and ratios), flux from one pool to another can be measured using the MT technique. MT is technically challenging compared to "standard" ^{31}P MRS. The basis of MT is selective saturation or inversion of a resonance of one moiety which undergoes chemical exchange to another. If the rate of exchange is fast compared to T1, then the saturation or inversion is transferred; quantification of exchange rates requires knowledge of the T1 and MT rate (120). MT can provide information on the flux between PCr and ATP and hence the rate of creatine kinase (121, 122). Similar methodology has also been applied to assess the flux between Pi and ATP to estimate ATP synthesis rate in brain (106, 145). However, concern surrounds this technique as ATP synthesis rates from MT transfer are significantly higher than the rates of oxidative ATP synthesis measured by other techniques, shown in muscle, heart, and liver (123, 124). This discrepancy is usually attributed to rapid Pi-ATP exchange *via* glycolysis, which can produce significantly higher MT measures of Pi → ATP flux compared with net oxidative Pi → ATP flux (123–126). Although this does not necessarily invalidate MT measures of ATP synthesis rates in brain (145, 127), where average measures agree with rates calculated indirectly from previously reported cerebral metabolic rate of glucose consumption (145), varying levels of anesthesia in TBI may also influence results.

Therapeutic and Prognostic Potential

^{31}P MRS studies have shown changes in PCr, Pi, pH, and Mg^{2+} in the brain following TBI, with ATP being relatively unaffected except for under extreme stress in experimental TBI, although timing of when after injury a ^{31}P MRS study is performed is key.

In a clinical setting, ^{31}P MRS cannot be performed in the hyperacute period after injury because of the time required transferring a patient to hospital and stabilizing them. However, if a patient displayed severely depressed PCr/Pi and pH measured by ^{31}P MRS on the day of injury, this may suggest that the initial injury was extreme, or compounded by a period of hypotension, which may or may not have been known about. As well as prompting meticulous control of cerebral perfusion pressure, causes for hypotension could be investigated if they were not already apparent.

The degree of PCr/Pi depression may also correlate with outcome, if performed on the day of injury, as seen in animal studies (107, 108, 111). However, there is a paucity of outcome data from ^{31}P MRS animal studies reporting changes in PCr or Pi performed more than 12–48 h after injury, in what would be a more achievable timeframe clinically. However, as mentioned above, the situation with human TBI patients seems to differ from animal studies, with human TBI causing a higher PCr/ATP or PCr/Pi ratio than healthy controls, and TBI resulting in a more alkaline brain pH when performed 4–21 days after injury. If the Pi peak is not distinguishable from baseline noise, PCr/ATP could be used as an alternative ratio. However, in the event of TBI with hypotension or extreme injury, an equivalent fall in both PCr and ATP species could (in principle) lead to no net change in their relative ratio (PCr/ATP) (102).

^{31}P MRS studies performed in the acute to subacute period after injury that display an elevation in the PCr/Pi and PCr/ATP ratios may represent neuroinflammatory changes in TBI (102), which merits further investigation. Further study is ongoing characterizing these changes and their pathophysiological basis.

Although brain free intracellular Mg^{2+} appears to be very sensitive to injury in the acute and subacute period following TBI, it does not easily distinguish between moderate and severe grades of injury. Whereas there may be a greater fall in Mg^{2+} following moderate–severe injury than mild injury, paradoxically there is no change following extreme injury (108).

A summary of the effect of TBI on metabolites interrogated by ^{31}P MRS is shown in **Table 3**.

^{13}C MRS

Hardware and Sensitivity

Whereas *in vivo* ^{1}H MRS measures brain metabolites by detecting the hydrogen atoms within these molecules, ^{13}C MRS does so by detecting the ^{13}C isotopes in their structure. ^{13}C MRS is much less sensitive than ^{1}H MRS as only 1.1% of naturally occurring carbon is the MR visible isotope ^{13}C and most organic molecules contain many more hydrogen atoms than they do carbon atoms. The Larmor (natural) frequency of ^{13}C is also a quarter of that of ^{1}H, so each atom releases much less energy when it relaxes to be detected by the scanner. These factors combine to give ^{13}C MRS a sensitivity of only 0.018% of that of ^{1}H MRS (15). Consequently, *in vivo* ^{13}C MRS studies are almost always performed with an infusion of ^{13}C enriched metabolites to boost the signal from the brain. Even so, large voxels are typically also used to acquire as much signal as possible.

Methods of Detection, Localization, and Decoupling

The sensitivity of ^{13}C can be further improved by various techniques that use the interactions of ^{1}Hs naturally bonded to the ^{13}C nuclei. Nuclear Overhauser enhancement (NOE) and polarization transfer are two different techniques for increasing signal that transfer some spin polarization from ^{1}H to ^{13}C. Proton decoupling is another important technique as J-coupling of

TABLE 3 | Summary of metabolite changes following traumatic brain injury (TBI) detectable with *in vivo* ^{31}P MRS.

	Changes in hyperacute stage[a]	Changes in subacute stage	Correlation to prognosis
Metabolites associated with energy metabolism	↓ PCr ↑ Pi ↓ PCr/Pi	↑ PCr/ATP ↑ PCr/Pi	 ✓*
Change in pH	↓		✓
Mg^{2+}	↓†	↑↓	✓*†

PCr, phosphocreatine; Pi, inorganic phosphate; ATP, adenosine triphosphate.
↑: increase in metabolite; ↓: decrease in metabolite; ✓: potential clinical use as a prognostic predictor.
**Animal studies.*
†In animal studies, Mg^{2+} falls proportionally to injury severity, except for following the most severe TBI.

^{13}C nuclei to their bonded ^{1}Hs causes splitting of metabolite peaks into complex patterns of small multiplets that can be difficult to interpret. This interaction can be broken using proton decoupling at the same time as applying either NOE or polarization transfer, further improving spectral resolution (15, 20).

As well as directly detecting the ^{13}C in a metabolite, ^{13}C MRS can be performed indirectly through detecting the effects of the ^{13}C on the ^{1}Hs that are bonded to it, termed ^{1}H-observe [^{13}C-edited] spectroscopy, or proton-observe carbon edited (POCE) spectroscopy. POCE increases the sensitivity even more than using the polarization transfer technique (almost to that of ^{1}H MRS) and directly provides ^{13}C fractional enrichment values (20). However, this increased sensitivity, comes with the narrow spectral range of the ^{1}H MRS scale (5 ppm) where many peaks overlap, so resolving individual peaks can be more difficult. Crowding of the spectra is much less of a problem with the great spectral range of direct ^{13}C MRS detection (200 ppm). Also, ^{13}C nuclei which do not have ^{1}H attached, such as the carboxylate carbon in Glu and Gln can be measured by direct ^{13}C MRS while they cannot be measured with the indirect (POCE) MRS technique.

Proton decoupling, NOE, polarization transfer or indirect detection require a ^{1}H channel in addition to the ^{13}C channel on the RF head coil (20). A complex sequence of pulses must be passed down each channel in quick succession which can result in current induction from one channel to the other, introducing further noise in the spectrum if appropriate arrangement of these coils with effective filtering is not observed.

The RF pulses for broadband proton decoupling deposit a significant amount of energy into the patient, causing tissue heating. The relevance of tissue heating depends on how thermally sensitive the tissue is, and by how much it is heated. The specific absorption rate limit to minimize heating of tissue is critical with regards to the eyes; so ^{13}C MRS with proton decoupling is typically performed using surface coils to address ROIs of the brain that avoid them.

Due to the abundance of carbon atoms in the long fatty acid chains of subcutaneous scalp lipids, non-localized ^{13}C spectra of the brain are dominated by large lipid peaks at 20–50 ppm. Glycerol that forms their lipid backbone also produces pronounced, but smaller, peaks at 63 and 73 ppm. These scalp peaks must typically be excluded with voxel selection or outer volume suppression for brain metabolites to be measured. Furthermore, the chemical shifts of some key metabolites place them within the lipid range of 20–50 ppm meaning that voxel selection must be rigorous. The high concentration of lipid in cerebral white matter does not pose the same problem as it is bound up tightly in myelin so its MR signal decays too rapidly for detection by *in vivo* ^{13}C MRS (128).

Glycogen

Despite glycogen's large size [10^6–10^7 Da (129)] it is freely mobile and found in the human brain at concentrations of 5 mM/kg in glia (130) so it is the only brain metabolite of interest visible on ^{13}C MRS natural abundance studies using reasonable scan times. It is thought to be 100% MR visible (131) with a peak at 100 ppm, split by bonded ^{1}Hs if ^{13}C spectra are acquired without proton decoupling. Brain glycogen measured by enzymatic extraction has been shown to increase in regions of focal injury after experimental TBI in rats compared to uninjured regions, but it is not known if this correlates with degree of histological injury or outcome (132).

Dynamic Studies of Glucose, Lactate, Glu, and Gln

Most ^{13}C MRS studies of human brain metabolism involve infusion of ^{13}C enriched metabolically active substrates and detection of that signal as it makes its way sequentially through different metabolic pools. The most commonly studied substrate in the brain is 1-^{13}C glucose: as it is infused it appears in the brain at 94 and 98 ppm (α and β isoforms). It is then metabolized to lactate by glycolysis (principally), producing a lactate peak at 22 ppm in the brain spectra. The ^{13}C label is then incorporated into the TCA cycle where it is spun out from α-ketoglutarate as Glu, detectable at 34 ppm. ^{13}C Glu is released from neurons and taken up by glia where it is converted into Gln (5), detected at 32 ppm. Using mathematical models and certain assumptions, the rate of brain glucose uptake can be calculated from the appearance of glucose, the rate of glycolysis from the appearance of lactate, the TCA cycle rate from the appearance of Glu and neuronal–astrocyte coupling by the appearance of Gln (5, 15, 133). Alternative labeling patterns of glucose can be used, such as 2-^{13}C and U-^{13}C6 glucose that share identical biological effects but produce different spectra. There are benefits and limitations for each (20). Dynamic ^{13}C studies can also be performed using ^{13}C acetate and ^{13}C beta-hydroxybutyrate. Acetate is predominantly metabolized by the glia, allowing the metabolic rates of this specific cell population to be measured (5, 20) whereas beta-hydroxybutyrate is predominantly metabolized by neurons during periods of fasting when it supplies 60% of the fuel for brain (5).

Positron emission tomography studies of brain metabolism using [^{18}F]-fluorodeoxyglucose can measure the brain's uptake and phosphorylation of glucose, but are unable to follow its metabolism further downstream: ^{13}C MRS measures glucose uptake, but also the TCA cycle rate and neuronal–glial coupling. Changes in the rate of the TCA cycle and Glu/Gln cycling have been reported following stroke, Alzheimer's disease, and diabetes mellitus (5). No ^{13}C infusion studies of human TBI have been reported to date, although the technique has potential to shed light on the effects of TBI on these key processes.

^{13}C Hyperpolarization

^{13}C hyperpolarization is a technique that transiently increases the signal from ^{13}C nuclei 10,000-fold (134), allowing detection of ^{13}C metabolites in a short timeframe. Without performing hyperpolarization, nuclear polarization is poor because the energy required to align a nuclear spin against a magnetic field is so small that thermal fluctuations can easily overpower these transitions despite using large magnetic fields. Although various hyperpolarization methods exist, the version implemented for clinical studies is dissolution dynamic nuclear polarization (DNP). The following description is from Nelson et al. (135). "Hyperpolarized ^{13}C MRI is a relatively new molecular imaging technique with an unprecedented gain in signal intensity of 10,000- to 100,000-fold (134) that can be used to monitor uptake and metabolism

of endogenous biomolecules (136, 137). The magnitude of the increase in sensitivity depends on the degree of polarization that is achieved, the T1 relaxation time of the ^{13}C agent, the delivery time, and the MR methods applied. Hyperpolarized agents are generated by mixing ^{13}C-labeled compounds with an electron paramagnetic agent (EPA), placing them in a 3.35-T magnetic field, cooling to ~1 K, and using microwaves to transfer polarization from the electron spin of the EPA to the ^{13}C nuclei of the biomolecule (13). Once the polarization has reached the required level, the sample is rapidly dissolved with hot, sterile water and neutralized to physiological pH, temperature, and osmolarity. Intravenous injection of the hyperpolarized solution and observation using ^{13}C MR allow its delivery and metabolic products to be monitored (15). The data must be obtained as rapidly as possible after dissolution because the enhancement decays at a rate determined by the T1 relaxation time of the agent, which is about 60 s for [1-^{13}C] pyruvate at 3 T. Translation of hyperpolarized technology into human subjects has been challenging because it requires specialized instrumentation to prepare the agent in a sterile environment, filter out the EPA, perform quality control, and rapidly deliver samples to the patient." DNP works best for metabolites with carboxylate carbons which have long T1 so polarization decays more slowly; clinical studies typically use [1-^{13}C] pyruvate, such as the first-in-human study that interrogated the metabolism of prostate cancer (135). To date, no ^{13}C hyperpolarization studies in human TBI brain have been published.

A ^{13}C hyperpolarization study of rat TBI has recently been performed by DeVience et al. using 1-^{13}C pyruvate (138). Controlled cortical impact of rat brain produced lower ^{13}C-bicarbonate signals and higher [1-^{13}C] lactate in traumatized regions of brain than non-traumatized brain. This correlated with cortical scarring and persisting cell death on histological analysis performed 30 days after injury. This suggests a shift from oxidative to non-oxidative metabolism due to TBI, in the absence of gross hypoperfusion, as no difference in [1-^{13}C] pyruvate signal was seen in the traumatized region. Surprisingly, sham-operated animals that underwent craniotomy, but no intentional cortical injury showed much less significant but similar changes to those exposed to cortical impact. In mice, a ^{13}C hyperpolarization study with 1-^{13}C pyruvate, performed 12 h after controlled cortical impact to brain showed an increase in the 1-^{13}C lactate/1-^{13}C pyruvate ratio detected with *in vivo* ^{13}C MRS in the injured hemisphere compared to the contralateral uninjured hemisphere (139).

Conventional (non-hyperpolarized) ^{13}C MRS studies that rely on the infusion of ^{13}C enriched substrates detect downstream metabolites of the substrates infused. Hyperpolarized studies are much more limited due to the very transient nature of hyperpolarization enhancement of ^{13}C MRS signal so only metabolites a few steps downstream can be detected before the hyperpolarized effect is lost. Hyperpolarization and conventional ^{13}C MRS labeling studies can be considered complementary as they address metabolic pathways on different timescales.

Summary of ^{13}C MRS and Clinical Role

Despite the potential of ^{13}C enriched steady-state infusion studies to shed light on the biochemistry of TBI, we do not currently see it as a routine clinical tool in the management of TBI, due to the extensive time required in the scanner for data acquisition, large volumes of expensive ^{13}C-labeled infusates required and complex post-acquisition analysis. However, conceivably ^{13}C isotope costs may come down in future, scanners become more sensitive, simpler data analysis strategies devised, and workarounds adopted such as starting the infusion outside of the magnet to reduce the time the patient is inside. Other possibilities are natural abundance (unlabeled) studies of brain glycogen that may show changes related to TBI, but few studies to date have demonstrated this and the scan times required are also long (20, 130).

Hyperpolarized ^{13}C MRS shows great potential in the monitoring of brain metabolism for the clinical management of TBI. The short acquisition time and clear signal it produces puts it on par with ^{1}H MRS, although ^{13}C hyperpolarization has the downside of expensive ^{13}C-substrate and hyperpolarization equipment, and the larger team of expert staff necessary. Metabolic derangement by elevated lactate/pyruvate or lactate/bicarbonate ratios can be mapped throughout the brain unlike techniques such as microdialysis, which only sample from a single region of brain which may miss key regions where brain energy is failing. As targeted therapies for brain injury become available they may be delivered focally to regions of metabolic dysfunction. *In vivo* ^{13}C hyperpolarization is still a relatively new technique so its development with further advances are expected.

PRACTICAL CONSIDERATIONS: MR CONDITIONAL EQUIPMENT AND RISKS

Taking critically ill patients with acute severe TBI for an MR study can be challenging; patients typically have multiple monitoring devices and require intensive support. However, with the use of MR conditional ventilators, syringe drivers, and an appropriate ICP monitor setup, a patient's critical care bed can effectively be recreated inside the MR suite.

Equipment MR compatibility is graded. Whereas plastic ventilator tubing is MR safe at any field strength and is called "MR Safe" a mechanical ventilator may be suitable to use at 3 T but not at 7 T: "MR conditional." Even if it is designed to be used with a 3 T scanner, often that allows it to be taken into the room, but not right up to the magnet bore where the magnetic field is strongest. Ventilator extension tubing must be prepared to reach the patient.

As well as the projectile risk of ferrous objects, an item's MR conditional status depends on its performance within the MR environment. Both the changing gradient magnetic fields used for localization and the power and frequency of the RF pulses can cause induction of current in non-ferrous metals. This is greatest when the length of the object, commonly a wire, is a multiple of the wavelength of the RF pulse (140). Furthermore, the ventilator or patient monitor can produce electromagnetic interference that will affect the image or spectra quality.

An important example of this for patients suffering from TBI is the commonly used Codman MircoSensor ICP Transducer (Codman & Shurtleff, Inc.). When using the body (main scanner) coil to transmit and a head coil to detect at 3 T, the electrical current that is induced is sufficient to heat the wire and damage

the probe. This necessitates replacement of the probe, and consideration of potential burns to the patient's skin and brain that are in contact with the wire. This effect can be stopped by looping the extra length of wire away from the patient's skin, which introduces a radiofrequency choke that limits current induction (141). This allows safe use of the microsensor in a 3 T scanner during MR data acquisition. Two other monitoring devices that are often used in the management of acute severe TBI cannot be used during an MRS study: brain tissue oxygen probes (such as Licox®) and microdialysis pumps. Licox catheters must be disconnected with their connecting lead but the attached intracranial probe can generally be left in place for reconnection after the study. Microdialysis catheters may similarly be left attached but the battery that drives the pump is MR unsafe, so must be removed. Whereas these two monitoring systems are useful for clinical management, a brief hiatus is rarely critically disruptive and probably outweighed by the information that MRI and MRS studies provide.

Other specific items that are a projectile risk in the static magnetic field are at risk of current induction causing burning or rotational injury due to changing magnetic fields include: pacemakers and their leads, ECG wires and dots, deep brain and spinal cord stimulation leads, patient oxygen cylinders, some cerebral aneurysm clips, and metal fragments in patients' eyes. If these are present and non-removable (such as an implanted pacemaker), they will preclude examination by MRS/MRI. In the acute period after a severe TBI, it is difficult exclude a history of a metal fragment in a patient's eyes, but in practice these would

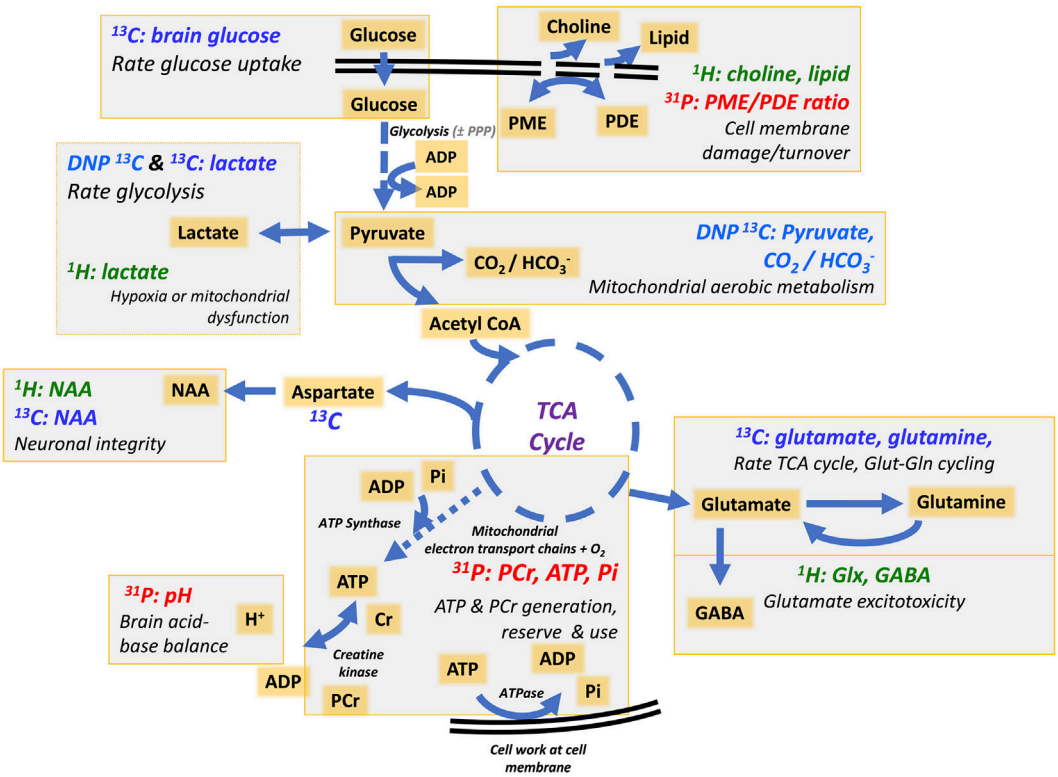

FIGURE 5 | Simplified schematic of different metabolites and processes in the brain that can be interrogated using ^{1}H MRS, ^{31}P MRS, ^{13}C MRS, and DNP ^{13}C MRS. ^{1}H and ^{31}P MRS show endogenous metabolites; ^{13}C MRS requires exogenous ^{13}C-enriched substrate, while for DNP ^{13}C MRS the exogenous ^{13}C-enriched substrate is hyperpolarized before administration, transiently boosting ^{13}C signal. Pathways include uptake of glucose that is metabolized *via* glycolysis in the cytosol [with a low yield of ATP per mole of glucose consumed] producing pyruvate. Pyruvate can enter mitochondria where it is converted into acetyl-CoA that enters the TCA cycle. Pyruvate remaining in the cytosol can be converted into lactate, simultaneously recycling NADH into NAD^{+} allowing glycolysis to continue. The rate of glucose uptake and glycolysis can be interrogated with ^{13}C MRS (glucose and lactate appearance) whereas the relative flux of "anaerobic" metabolism vs. aerobic mitochondrial metabolism can be measured with DNP ^{13}C MRS (lactate vs. HCO_3^-) and ^{1}H MRS (lactate). The TCA cycle drives the mitochondrial electron transport chain for high-yield ATP synthesis. The rate of the TCA cycle can be calculated by the rate of appearance of ^{13}C labeled glutamate (Glu) (^{13}C MRS) and ATP produced measured with ^{31}P MRS (γ-ATP, β-ATP, and Pi). Neuronal integrity and mitochondrial function can be measured indirectly by detection of NAA with ^{1}H MRS (and ^{13}C MRS). Neuronal–glial coupling is represented by Glu–glutamine (Gln) cycling detected by ^{13}C MRS, whereas total combined Glu and Gln that may be raised in pathological excitotoxicity can be measured with ^{1}H MRS. Cell membrane integrity and damage and turnover may be represented by ^{1}H MRS (choline and lipid) and ^{31}P MRS (PME/PDE ratio), which also can detect the balance and consumption of high-energy phosphates (ATP, PCr, and Pi). Further details of the above, and other MRS-detectable molecules (including creatine, myoinositol, glycogen, and nicotinamide-adenine dinucleotides), can be found in the text. Abbreviations: ADP, adenosine diphosphate; ATP, adenosine triphosphate; Cr, creatine; DNP, dissolution dynamic nuclear polarization; GABA, gamma-aminobutyric acid; NAA, *N*-acetylaspartate; MRS, magnetic resonance spectroscopy; NAD^{+}, nicotinamide adenine dinucleotide oxidized form; NADH, nicotinamide adenine dinucleotide reduced form; PCr, phosphocreatine; PDE, phosphodiester; PME, phosphomonoester; Pi, inorganic phosphate; PPP, pentose phosphate pathway; TCA, tricarboxylic acid.

have been detected or excluded on CT examination at presentation for acute TBI. Some tattoos and permanent eyeliners may also be heated by the RF pulses but these are often not an absolute contraindication to examination by MR. The issue of guarding against tissue heating is not just confined to metal fragments but also to uncontaminated tissue and has been mentioned above.

Head coils that completely envelope the head make it difficult accessing the patient's airway in an emergency. Head coils with joins that can open-up, either hinged along one side, or else with a front half that can be detached completely (see **Figure 4B**), allow access to the airway in an emergency and make correctly positioning the patient's head within the head coil easier. This is even more relevant when the patient has prominent intracranial monitoring. A potential obstacle to performing MR studies on patients with acute severe TBI is the lack of head elevation that can be achieved during the scan. This is even more restricted by volume head coils. Head elevation to 30° is an effective initial treatment step in the management of raised ICP (142), but only up to 5° of head elevation can be achieved with padding inside a head coil. Patients with very brittle raised ICP on maximum therapy must wait before an MRS study can be performed if they will not tolerate any period lying flat.

SUMMARY, CONCLUSION, AND FUTURE PROSPECTS

^{1}H, ^{31}P, and ^{13}C *in vivo* MRS are complementary techniques that allow non-invasive measurement of different aspects of brain metabolism that may contribute to the clinical management of patients with acute severe TBI (see **Figure 5**).

^{13}C MRS measures "upstream" brain energy metabolism: the breakdown of infused ^{13}C-labeled glucose (or other sugars) *via* glycolysis and the TCA cycle. To date, few studies of ^{13}C MRS in TBI exist, but the development of *in vivo* hyperpolarized techniques shows promise in this field. ^{31}P MRS allows measurement of "downstream" metabolism by detecting high-energy phosphates (ATP and PCr) produced by oxidative phosphorylation and creatine kinase in mitochondria. Changes in these metabolites have been noted in a few human and animal studies of TBI but further study is required.

^{1}H MRS is the most commonly used MRS technique for studying brain metabolism following TBI. It has the potential to measure various metabolites: some are associated with "upstream" brain energy metabolism such as lactate, Glu and Gln, whose flux can also be measured by ^{13}C MRS. Creatine and NAA are associated with the "downstream" metabolism of ATP and PCr, which can also be measured with ^{31}P MRS. Free brain lipid and choline are not as directly linked to brain metabolism and are likely markers of cell membrane damage. ^{1}H MRS has shown great potential as an additional prognostic tool for patients with acute severe TBI, but the region of the brain that should be studied and how long after injury it should be performed is debatable (143). The development of standardized protocols of acquisition and analysis would facilitate its progression into clinical care.

Magnetic resonance spectroscopy has potential to play a bigger role in Phase II trials of therapies by providing surrogate markers and "tissue fate" measures that can help determine efficacy and inform whether a larger Phase III trial would be worthwhile or not.

Finally, the non-invasive (or minimally invasive) nature of MRS makes it an ideal technique for follow-up of patients' post-TBI. There is evidence to suggest that TBI produces long-term changes in the brain and that neurodegeneration occurs, with earlier onset of pathologies such as Parkinson's and Alzheimer's diseases (144). Better understanding of brain biochemistry may help development of better therapies. MRS is uniquely placed to shed light in such investigations.

AUTHOR CONTRIBUTIONS

MS, JLY, and KC designed the review. MS, JLY, KC, AS, and MM drafted the manuscript. All the authors reviewed, edited, and approved the manuscript.

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The Correlation between Cerebral Blood Flow Measured by Bedside Xenon-CT and Brain Chemistry Monitored by Microdialysis in the Acute Phase following Subarachnoid Hemorrhage

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Cerebral microdialysis (MD) may be used in patients suffering from subarachnoid hemorrhage (SAH) to detect focal cerebral ischemia. The cerebral MD catheter is usually placed in the right frontal lobe and monitors the area surrounding the catheter. This generates the concern that a fall in cerebral blood flow (CBF) and ischemic events distant to the catheter may not be detected. We aimed to investigate if there is a difference in the association between the MD parameters and CBF measured around the MD catheter compared to global cortical CBF and to CBF in the vascular territories following SAH in the early acute phase. MD catheter was placed in the right frontal lobe of 30 SAH patients, and interstitial glucose, lactate, pyruvate, glycerol, and lactate/pyruvate ratio were measured hourly. CBF measurements were performed during day 0–3 after SAH. Global cortical CBF correlated strongly with CBF around the microdialysis catheter (CBF-MD) ($r = 0.911$, $p \leq 0.001$). This was also the case for the anterior, middle, and posterior vascular territories in the right hemisphere. A significant negative correlation was seen between lactate and CBF-MD ($r = -0.468$, $p = 0.009$). The same relationship was observed between lactate and CBF in anterior vascular territory but not in the middle and posterior vascular territories. In conclusion, global CBF 0–3 days after severe SAH correlated strongly with CBF-MD. High lactate level was associated with low global CBF and low regional CBF in the right anterior vascular territory, when the MD catheter was placed in the right frontal lobe.

Keywords: cerebral blood flow, microdialysis, lactate, Xenon-CT, subarachnoid hemorrhage

INTRODUCTION

Today multimodal monitoring is a part of the neurointensive care (NIC) management of patients suffering severe subarachnoid hemorrhage (SAH) (1). Microdialysis (MD) of the extracellular fluid may be used to monitor the metabolic state of the tissue in order to detect secondary injuries such as ischemia (2, 3). Cerebral ischemia is a feared complication which occurs in 20–30% of patients suffering from SAH and increases the morbidity and mortality (4). Monitoring cerebral metabolites

and cerebral blood flow (CBF) provides vital information on tissue at risk of developing ischemia. However, MD is a focal technique that measures a small region of the brain tissue, and it is recommended that, if possible, the MD catheter should be placed in the vascular territory at risk (5). At our department, the MD catheter is routinely placed in right frontal lobe. This is based on the assumption that both the middle cerebral artery (MCA) and the anterior cerebral artery (ACA) territories will be monitored. Bedside Xenon-CT is used routinely in our NIC unit in order to assess the regional CBF in patients following SAH (6–8). In a previous Xenon-CT study including 64 SAH patients, we could not find any correlation between regional CBF and aneurysm location (7).

Earlier studies in SAH patients using positron emission tomography simultaneously with MD have shown increased levels of energy metabolites (9) and glutamate (10) under conditions with low CBF.

The objective of the current study, using bedside Xenon-CT, was to investigate if there is a difference in the association between the MD parameters and CBF measured around the MD catheter compared to global cortical CBF and to CBF in the vascular territories during the early acute phase of SAH.

MATERIALS AND METHODS

Study Population and Study Design

Thirty patients with SAH who were admitted to the NIC unit, Section of Neurosurgery, Uppsala University Hospital, between October 2012 and May 2015 were included in the study.

The inclusion criteria were patients who underwent a Xenon-CT at day 0–3 after onset of SAH and received a MD catheter at admission. These patients needed to be mechanically ventilated for the Xenon-CT and in need of a ventriculostomy for simultaneous insertion of a MD catheter. Patients with a preexisting neurological deficit, an SAH resulting from trauma, or arteriovenous malformation were excluded. The SAH was verified by CT scanning and the aneurysm was visualized by a CT angiography and/or digital subtraction angiography (6).

Neurointensive Care

The standardized protocol at our NIC unit, which is well described previously (6, 11), is based on intensive physiological monitoring and aggressive therapy of any derangement to avoid or minimize secondary brain injury. Unconscious patients are mechanically ventilated and receive a ventriculostomy. If ICP is above 20 mmHg, the drainage system is opened and cerebrospinal fluid drained against a pressure level of 15 mmHg. Hypotension is treated first with albumin 20% and crystalloid solutions, and with Dobutamine (Algol Pharma AB, Kista, Sweden) if needed. The goal is to keep CPP above 60 mmHg. Identified aneurysms are treated early by endovascular coiling or surgical clipping. All patients receive nimodipine (Nimotop®, Bayer AB, Solna, Sweden).

CBF Measurements

At our department, bedside Xenon-CT has been introduced as a routine and is performed on patients with SAH and mechanically ventilated at day 0–3, day 4–7 and after 7 days after admission as far as the necessary resources are available (6). This time point is used since delayed cerebral ischemia (DCI) is rarely seen before day 3 following onset of SAH (12, 13). The principal of CBF measurements using Xenon-CT has been previously described by Yonas et al. (14–16), and the procedure used at our department has also been previously described (6). Briefly, a concentration of 28% of stable Xenon is administered to the patients breathing circuit for about 4 min using the Enhancer 3000 and the specially developed computer software (Diversified Diagnostic Products Inc., Houston, TX, USA). During the Xenon inhalation, eight CT scans at four different levels with 10-mm spacing are obtained by the CereTom® (Neurologica, Boston, MA, USA). The first CT-scan is adjusted using the scout view in order to avoid inclusion of coil artifacts. The computer software synchronizes the Xenon delivery and the CT scans. The resulting radiologic tissue enhancement of the Xenon wash-in enables CBF (ml/100 g/min) to be calculated and plotted as colored maps.

Mean blood flow in each of 20 evenly distributed cortical regions (ROIs) was calculated for each level, and the global CBF is given as a mean of all four levels. The vascular territories were analyzed as following: ACA—ROI 1–2 (right) and 19–20 (left), MCA—ROI 3–8 (right) and 13–18 (left), and posterior cerebral artery (PCA)—ROI 9–10 (right) and 11–12 (left) (**Figure 1**). The tip of the MD catheter was identified on the structural CT scans and an ROI was drawn manually (diameter = 3 cm) for the corresponding area around the MD catheter on the CBF scans. Territories with CT-defined hematoma or artifacts were noted and excluded.

Cerebral MD

The cerebral MD technique in NIC has previously been extensively used and described (2, 9). The intracerebral MD catheter is placed in the right frontal lobe cortex through a separate burr hole, anterior to the ventricular drain. For intracerebral MD monitoring, a 70 brain MD catheter is used (M Dialysis AB, Stockholm, Sweden) with a membrane length of 10 mm and a membrane cutoff of 20,000 Da. The catheters are perfused with artificial CSF (NaCl 147 mmol/l, KCl 2.7 mmol/l, $CaCl_2$ 1.2 mmol/l, $MgCl_2$ 0.85 mmol/l) (perfusion fluid CNS; M Dialysis AB).

The perfusion rate was measured as 0.3 μl/min using a microinjection pump (CMA-106, M Dialysis AB). MD urea was monitored to validate catheter performance (17). The MD samples were collected on an hourly basis. For the correlation analysis, the MD sample was collected at the end of the Xenon-CT exanimation. Interstitial glucose, lactate, pyruvate, glutamate, glycerol, and urea were analyzed enzymatically using a CMA 600 analyzer or ISCUS Clinical Microdialysis Analyzer (M Dialysis AB).

Statistical Analysis

All analyses were performed using SPSS Statistics for Macintosh, Version 23.0 (IBM®, Armonk, NY, USA). In order to assess the normality of the data set, the skewness and kurtosis of the distribution were analyzed. Since the parameters were not normally

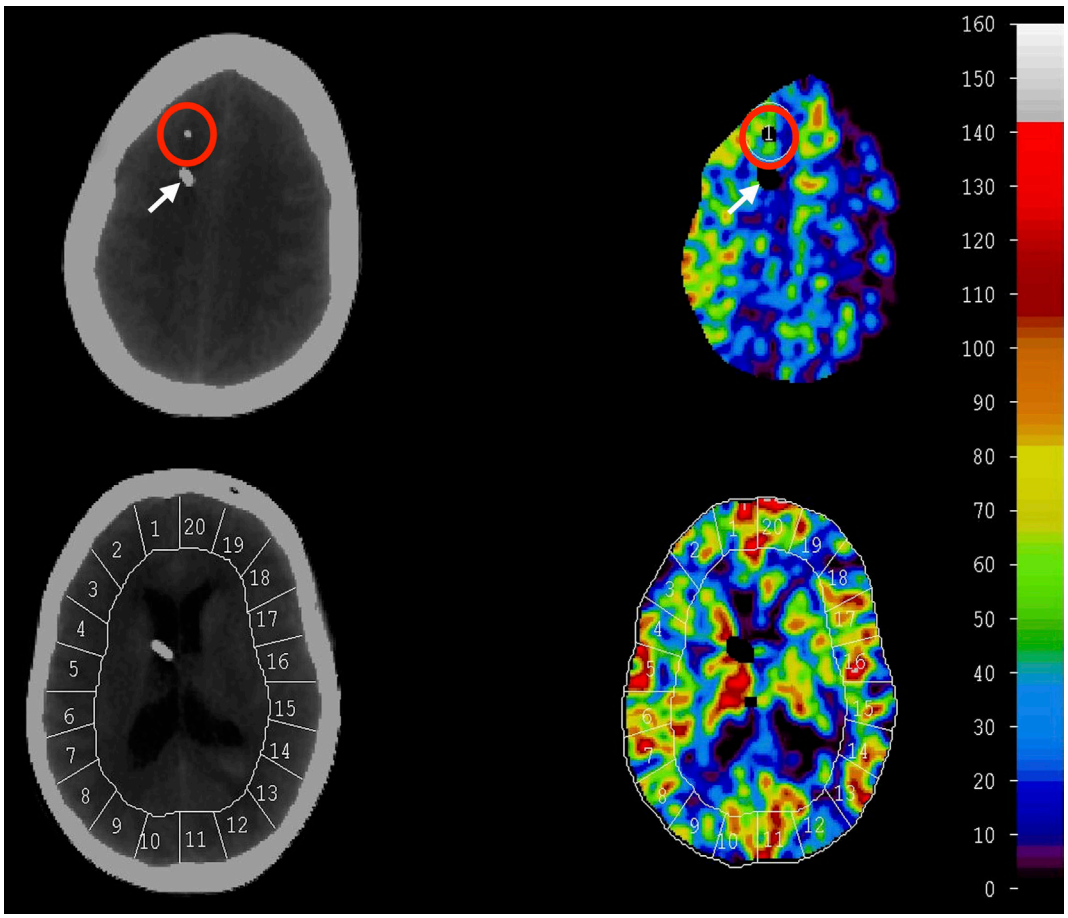

FIGURE 1 | Xenon-CT scans at different levels obtained by bedside mobile CT-scanner. Conventional CT images are obtained for evaluation and identification of microdialysis (MD) catheter. Following Xenon delivery tissue enhancement of the Xenon wash-in enabled cerebral blood flow (CBF) (ml/100 g/min) to be calculated and plotted as colored maps. Scale of CBF is ml/100 g/min and is given to the right. Twenty cortical ROIs were used for CBF calculation and regional vascular territory was identified (anterior cerebral artery 1–2, 19–20, medial cerebral artery 3–8, 13–18, posterior cerebral artery 9–10, 11–12). CBF around the MD catheter was calculated by drawing an ROI manually around the catheter (circle in red). White arrow indicates EVD.

distributed, Spearman's correlation was used. Bonferroni correction was performed for multiple comparisons. Results are expressed as mean ± SD and range within brackets. A p value <0.05 was considered statistically significant.

Ethics

The Uppsala University Regional Ethics Review Board for clinical research granted permission to undertake the study. Written informed consent was obtained from all patients or their proxy for study participation. The study was also approved by the local Radiation Safety Authority.

RESULTS

Demography and Clinical Data

Thirty patients with severe SAH were included, 5 males and 25 females. Demographics and clinical data including the distribution of aneurysm location are given in **Table 1**. The physiological parameters were stable during CBF measurements, and baseline values are shown in **Table 2**.

CBF Measurements

The mean cortical global CBF for all patients was 33.3 ± 13.5 ml/100 g/min (13.7–73.8) and CBF-MD was 30.3 ± 12.5 ml/100 g/min (11.0–56.9). There was a significant positive correlation between the global CBF and CBF-MD ($r = 0.911$, $p \leq 0.001$).

The correlation between CBF around the MD catheter and different vascular territories of the right hemisphere was calculated and showed to be significant ($p \leq 0.001$) (**Figure 2**). Stronger correlation was seen between CBF in the ACA territory and MD.

MD Results

There were no complications associated with MD catheter insertion. The MD data corresponding to the time of CBF measurements are shown in **Table 3**. There was a huge variation between the patients, in particular in glycerol and glutamate.

TABLE 1 | Demographics and clinical data including the distribution of aneurysm location.

Patient characteristics	*n* (%)
Sex	
Female	25 (83)
Male	5 (16)
Age (year)	58.9 (28–84)
Hunt and Hess at admission	
H&H I–II	6 (20)
H&H III	8 (26)
H&H IV–V	16 (53)
Fisher grade	
1–2	0 (0)
3	7 (23)
4	23 (76)
Aneurysm location	
AComA	9 (30)
ICA	4 (13)
PComA	5 (16)
MCA	6 (20)
AChA	1 (3)
PCA	1 (3)
BA	1 (3)
PICA	2 (6)
Unknown	1 (3)
Treatment	
Clip	4 (13)
Coil	25 (83)

Age is given as mean and (range).
AcomA, anterior communicating artery; ICA, internal carotid artery; PComA, posterior communicating artery; MCA, middle cerebral artery; AChA, anterior choroidal artery; PCA, posterior cerebral artery; BA, basilar artery; PICA, posterior inferior cerebellar artery.

TABLE 2 | Physiological parameters before and after the Xenon-CT measurements.

	Before	After
PaO_2 (kPa)	13.3 ± 2.8	13.6 ± 3
$PaCO_2$ (kPa)	5.1 ± 0.4	5.2 ± 0.5
FIO_2 (%)	39.3 ± 10.3	39.6 ± 10.1
MAP (mmHg)	94.1 ± 14.1	90.5 ± 11.8
ICP (mmHg)	17 ± 5	17.5 ± 4.7
CPP (mmHg)	77.6 ± 15.5	76.9 ± 13.1

Values are given as mean ± SD.
PaO_2, arterial partial pressure of oxygen; $PaCO_2$, arterial partial pressure of carbon dioxide; FIO_2, fraction of inspired oxygen; MAP, mean arterial blood pressure; ICP, intracranial pressure; CPP, cerebral perfusion pressure.

Pyruvate showed a strong positive correlation with lactate which remained significant after Bonferroni correction ($r = 0.738$, $p \leq 0.001$) and glucose ($r = 0.496$, $p = 0.006$), but the correlation with glucose did not pass the Bonferroni correction level. Glutamate showed the strongest correlation with lactate although not significant ($r = 0.375$, $p = 0.049$).

CBF and MD Correlation

Microdialysis parameters obtained at the time of Xenon-CT were compared to global cortical CBF and CBF-MD to investigate if there was an association between the CBF and the interstitial chemistry.

A significant negative correlation could be seen between lactate and CBF-MD ($r = -0.468$, $p = 0.009$). Lactate also negatively correlated with global CBF ($r = -0.408$, $p = 0.025$) but this did not remain significant following Bonferroni correction. There was a weak negative and non-significant correlation between L/P ratio and CBF-MD ($r = -0.364$, $p = 0.048$) and global CBF ($r = -0.329$, $p = 0.075$). No significant correlation could be found between CBF and glucose, pyruvate, glycerol, and glutamate.

The association between CBF in each vascular territory in the right hemisphere and MD parameters was investigated (**Table 4**). Lactate showed a significant negative correlation with CBF in ACA in the right hemisphere. This correlation was weaker and non-significant for CBF in PCA territory (**Figure 3**). After Bonferroni correction, only lactate correlated significantly with CBF in ACA territory.

DISCUSSION

In this study, we found that bedside monitoring of CBF using Xenon-CT in combination with MD in patients with SAH was feasible and safe. Placement of the MD catheter in the right frontal lobe following SAH showed a strong negative correlation between lactate and regional CBF in the anterior vascular territory but not in the middle and the posterior vascular territories. Few studies have combined CBF measurements with MD parameters in SAH patients (9, 10, 18). Using PET, Enblad et al. found that lactate, L/P ratio, and glutamate had the highest sensitivity for detecting ischemia in the area of the MD catheter (9). Also, Sarrafzadeh et al. found highest sensitivity for lactate and glutamate to detect ischemia using PET (10, 18).

High lactate levels have been reported to be associated with ischemia both in SAH patients and patients with head injury (19, 20). However, high lactate levels may also indicate hyperglycolysis (21, 22), explaining its rather low specificity as a biomarker of ischemia (9). Consequently, additional parameters such as L/P-ratio, pyruvate, and CBF should be evaluated to distinguish between ischemia, hyperglycolysis, and mitochondrial dysfunction (5, 23–25). In the current study, there was a significant negative correlation between CBF and lactate. However, the L/P ratio was not significantly correlated with CBF. We have recently reported on high lactate and low CBF during the acute phase following SAH in patients who later developed DCI (26). Different studies report on different levels of CBF thresholds for ischemia. Previous studies using Xenon-CT have reported on cortical CBF in awake normal subjects to be 52 ± 10 ml/100 g/min (27). In an additional study, comatose patients following head injury were compared to normal subjects and CBF threshold of 55.3 ml/100 g/min was defined as hyperemia (28). Our recent report on patients suffering severe SAH showed that patients who later develop DCI have initial low CBF levels of 23.7 ml/100 g/min compared to 37.5 ml/100 g/min in those who do not develop DCI (26). Current results are in line with previous findings and emphasize the important role of lactate in correlation with CBF in patients suffering SAH.

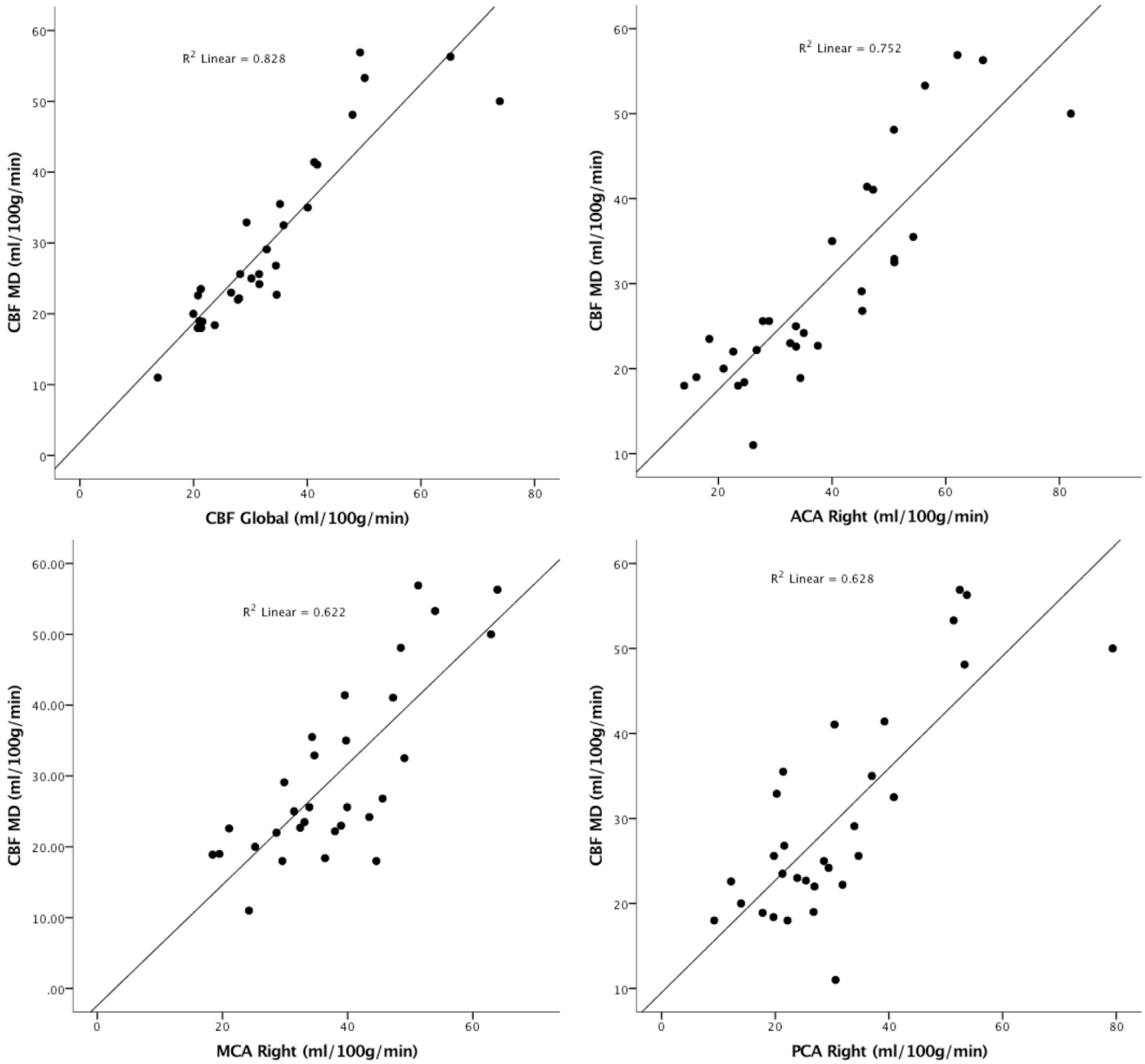

FIGURE 2 | The correlation between global cerebral blood flow (CBF) and CBF around the microdialysis catheter (CBF-MD) and different vascular territories in the right hemisphere.

TABLE 3 | Microdialysis data for all patients (n = 30) at the time of cerebral blood flow measurements.

	Glucose (mmol/l)	Lactate (mmol/l)	Pyruvate (μmol/l)	Glycerol (μmol/l)	Glutamate (μmol/l)	L/P ratio
Mean ± SD	2.3 ± 1.1	3.9 ± 2	143.6 ± 46.8	147.5 ± 149.3	32.2 ± 57.7	27.6 ± 11.8
Range	0.5 – 5.3	1.4 – 10.4	66.7 – 249.4	18 – 577	0.2 – 170	15.2 – 71.2

It is recommended that in patients suffering SAH, the MD catheter, if possible, should be placed in the vascular territory at risk (5). However, at our department, the MD catheter is placed in the right frontal lobe if there are no hematomas or infarction in conjunction to the ventriculostomy. This is based on the assumption that anterior brain a sensitive zone vascularized both by ACA and MCA, watershed areas, would offer an early warning signal of hypoperfusion and development of ischemia. In addition, this approach is logistically more feasible for the neurosurgeon on-call. In this study, we investigated how well the CBF in different vascular territories in the right hemisphere correlated with global CBF and if low CBF in different vascular

territories was correlated to pathological findings from MD placed in right frontal lobe. We found a strong and significant correlation between global CBF and all three vascular territories in the right hemisphere. However, pathological values indicated by high lactate were correlated with ACA territory but not with MCA and PCA territories. This may be as expected since these territories are less covered by a catheter placed in the right frontal lobe.

TABLE 4 | Correlation between cerebral blood flow (CBF) measurements in different vascular territories of right hemisphere and microdialysis parameters.

	Right ACA—CBF		Right MCA—CBF		Right PCA—CBF	
	r	*p*-Value	*r*	*p*-Value	*r*	*p*-Value
Glucose (mmol/l)	0.004	0.982	0.161	0.402	0.149	0.441
Pyruvate (μmol/l)	−0.363	0.049	−0.355	0.054	−0.120	0.526
Glycerol (μmol/l)	−0.026	0.897	0.007	0.971	0.119	0.545
Glutamate (μmol/l)	−0.324	0.093	−0.245	0.141	−0.067	0.736
Lactate (mmol/l)	−0.482	0.007[a]	−0.460	0.010	−0.242	0.198
L/P ratio	−0.354	0.055	−0.229	0.223	−0.348	0.060

The r-value is the Spearman correlation coefficient.
CBF, cerebral blood flow; L/P ratio, lactate/pyruvate ratio; ACA, anterior cerebral artery; MCA, middle cerebral artery and PCA, posterior cerebral artery.
[a]*Significant correlation after Bonferroni correction for multiple comparisons.*

The CBF measurements in this study were performed during day 0–3 following onset of SAH. Further studies are needed to evaluate the association between CBF and MD parameters at later time points after SAH with the catheter in right frontal lobe, given the increased risk of vasospasm and delayed focal cerebral ischemia. Another methodological limitation is the potential influence of artifacts from the EVD and MD catheter that may give inaccurately low CBF levels. This problem could not be avoided completely but is probably of minor magnitude. The artifacts were very small and comprised a minor proportion of the ROI volume analyzed.

A limitation of Xenon-CT CBF measurement compared to other methods such as PET is lower resolution and that only CBF can be quantified. However, PET is a complex and costly procedure with a need of cyclotron, while bedside Xenon-CT is more economical and accessible imaging technique with few adverse effects that can be used in the routine NIC to measure CBF.

In conclusion, the results of this study, using bedside Xenon-CT day 0–3 after SAH with simultaneous MD monitoring, show correspondence between high lactate levels and low regional CBF in the territory of right ACA but not in the middle and posterior vascular territories, when the MD catheter is placed in the right frontal lobe.

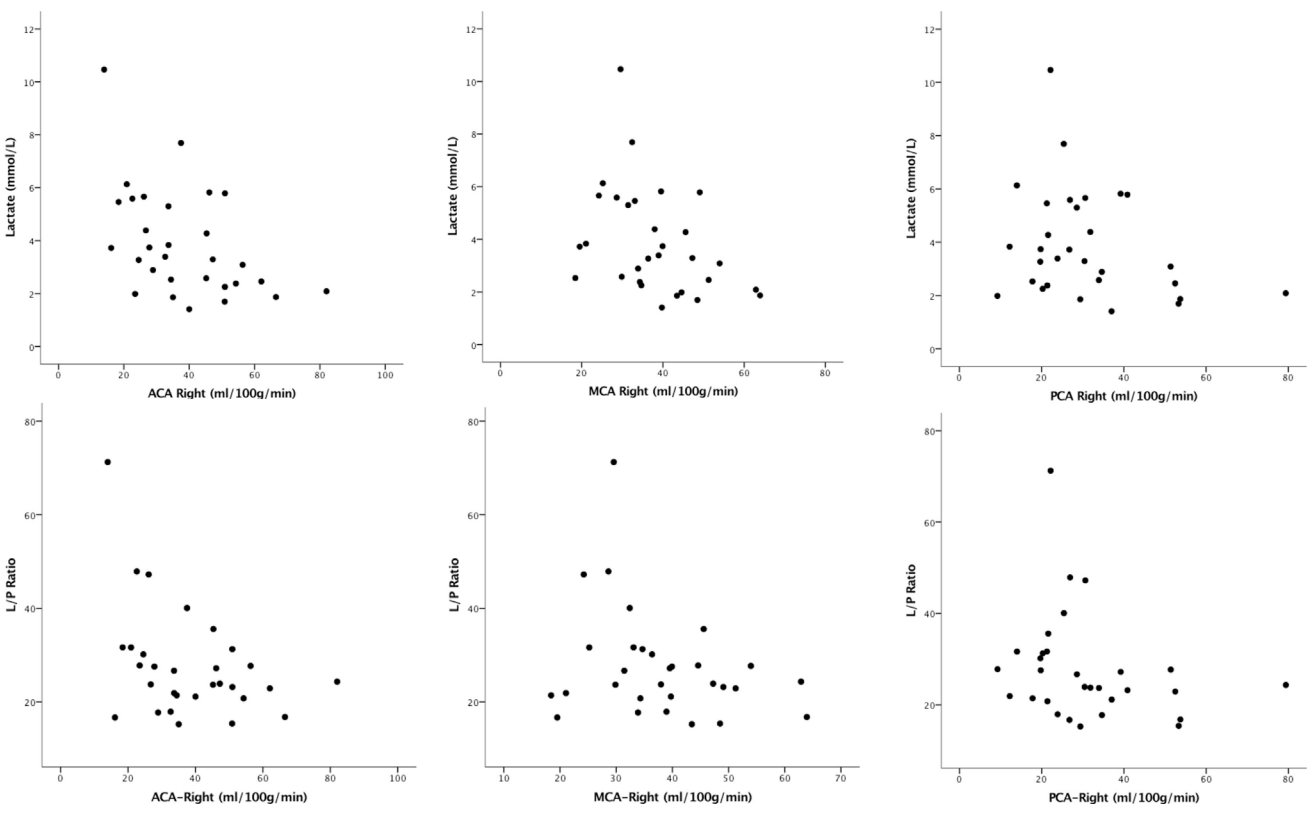

FIGURE 3 | The correlation between cerebral blood flow (CBF) in each vascular territory of the right hemisphere and microdialysis lactate/pyruvate ratio and lactate is shown. Lactate showed a significant correlation with CBF in anterior cerebral artery (ACA) territory in the right hemisphere.

ETHICS STATEMENT

The Uppsala University Regional Ethics Review Board for clinical research granted permission to undertake the study. Written informed consent was obtained from all patients or their proxy for study participation. The study was also approved by the local Radiation Safety Authority.

AUTHOR CONTRIBUTIONS

ER: design, data acquisition, analysis, and manuscript preparation. HE: Xenon-CT performance and manuscript preparation. TH, ER-E, and AL: data acquisition. PN: manuscript preparation. LH: data acquisition and manuscript preparation. PE: design and manuscript preparation.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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ORIGINAL RESEARCH
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Assessment of Platelet Function in Traumatic Brain Injury—A Retrospective Observational Study in the Neuro-Critical Care Setting

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Background: Despite seemingly functional coagulation, hemorrhagic lesion progression is a common and devastating condition following traumatic brain injury (TBI), stressing the need for new diagnostic techniques. Multiple electrode aggregometry (MEA) measures platelet function and could aid in coagulopathy assessment following TBI. The aims of this study were to evaluate MEA temporal dynamics, influence of concomitant therapy, and its capabilities to predict lesion progression and clinical outcome in a TBI cohort.

Material and methods: Adult TBI patients in a neurointensive care unit that underwent MEA sampling were retrospectively included. MEA was sampled if the patient was treated with antiplatelet therapy, bled heavily during surgery, or had abnormal baseline coagulation values. We assessed platelet activation pathways involving the arachidonic acid receptor (ASPI), $P2Y_{12}$ receptor, and thrombin receptor (TRAP). ASPI was the primary focus of analysis. If several samples were obtained, they were included. Retrospective data were extracted from hospital charts. Outcome variables were radiologic hemorrhagic progression and Glasgow Outcome Scale assessed prospectively at 12 months posttrauma. MEA levels were compared between patients on antiplatelet therapy. Linear mixed effect models and uni-/multivariable regression models were used to study longitudinal dynamics, hemorrhagic progression and outcome, respectively.

Results: In total, 178 patients were included (48% unfavorable outcome). ASPI levels increased from initially low values in a time-dependent fashion ($p < 0.001$). Patients on cyclooxygenase inhibitors demonstrated low ASPI levels ($p < 0.001$), while platelet transfusion increased them ($p < 0.001$). The first ASPI ($p = 0.039$) and TRAP ($p = 0.009$) were significant predictors of outcome, but not lesion progression, in univariate analyses. In multivariable analysis, MEA values were not independently correlated with outcome.

Conclusion: A general longitudinal trend of MEA is identified in this TBI cohort, even in patients without known antiplatelet therapies. Values appear also affected by platelet

inhibitory treatment and by platelet transfusions. While significant in univariate models to predict outcome, MEA values did not independently correlate to outcome or lesion progression in multivariable analyses. Further prospective studies to monitor coagulation in TBI patients are warranted, in particular the interpretation of pathological MEA values in patients without antiplatelet therapies.

Keywords: traumatic brain injury, progressive hemorrhagic injury, platelet aggregation, platelet aggregation inhibitors, multiple electrode aggregometry, cyclooxygenase inhibitors

INTRODUCTION

Traumatic brain injury (TBI) is a worldwide leading cause of mortality and disability (1). Following the acute phase, TBI is characterized by the development of secondary injuries (1), of which one is trauma-induced coagulopathy (TIC) (2). In severe TBI, the incidence of coagulopathy exceeds 60% (2) and is associated with lesion progression, mortality (3), and poor outcome (2). Alarmingly, despite conventional coagulation parameters within normal reference intervals, radiologic progression of intracranial lesions still occurs among more than 30% of patients (4). Moreover, the TBI setting now comprises increasingly older patients (5) for which antiplatelet therapy is common (6, 7), but associated with lesion progression and unfavorable outcome (7, 8). Consequently, hemorrhagic progression is the leading cause of seemingly preventable death (9) following TBI. In aggregate, there is considerable interest in diagnosis and treatment of coagulopathy in TBI patients, especially in the case of anticoagulative therapies.

Diagnosis of coagulopathy is complex (2, 10). Routine platelet and coagulation tests comprising platelet count, activated partial thromboplastin time (APTT), and international normalized ratio (2, 11) have been shown to be insufficient to diagnose the full perspective of coagulopathies (4). Similarly, more advanced laboratory tests, including thromboelastography and rotational thromboelastometry, do not adequately predict coagulopathy (10). Of the aforementioned, platelet count is commonly used to assess primary hemostasis and risk for bleeding progression, but platelet count is a quantitative measurement that does not account for platelet functionality (11). This has led to the development of platelet function assessment techniques (12). Among the methods described in Ref. (12), Multiplate® (Roche Diagnostics, Basel, Switzerland) and VerifyNow® (Accumetrics, San Diego, CA, USA) correlate with light transmittance aggregometry, the gold standard method for assessing platelet function (12).

While these techniques are primarily used to assess platelet function in cardiology patients, Multiplate®, based on multiple electrode aggregometry (MEA), use was shown to improve outcome prediction in a general trauma population (13). However, in TBI cohorts, the utility of MEA remains to be convincingly shown. In spite of this, MEA is used with increasing frequency in clinical practice, demonstrating the clinicians' need to evaluate platelet function and improve coagulation assessment within the field of TBI.

In our department, a majority of patients with TBI in need of neurointensive care unit (NICU) care is assessed using MEA. This presents a unique opportunity to evaluate its utility within the clinical setting. With this in mind, we sought to characterize MEA alterations following TBI, analyze how MEA levels are altered in drug induced coagulopathy, examine how MEA levels are associated with bleeding progression in patients with severe TBI, and how it subsequentially affects outcome.

MATERIALS AND METHODS

This was a retrospective observational study undertaken at the NICU at Karolinska University Hospital (Stockholm, Sweden) including patients treated between February 2010 (clinical introduction of MEA) and May 2014. The work was approved by the local ethics committee in Stockholm County, the Central Ethical Review Board (diary numbers 2014/1488-31/5 and 2015/1675-31/1).

Study Criteria

Patients were included if they had suffered a traumatic intracranial lesion, were of ≥15 years of age, and had been admitted to the NICU during the years 2010–2014. All patients included were at all times treated according to local routine at the NICU, as earlier described in Ref. (14).

Definitions

Trauma severity was assessed using the definitions of the Advanced Trauma and Life Support system (15) and is described as Injury Severity Score (ISS) (16). CT scans were evaluated by a trained examiner, blinded from coagulation measurements and outcome. Radiological parameters included midline shift (in mm), traumatic subarachnoid hemorrhage, epidural hematoma, acute subdural hematoma, and cerebral contusions. CT scans were graded according to Ref. (17), Rotterdam CT score (18), and Stockholm CT score (19). Stockholm CT score was used in the models as it has been shown to be the more accurate outcome predictor of these (20). The admission and follow-up CT scans were evaluated with regard to radiologic intracranial hemorrhagic progression. All types of intra- and extra-parenchymal lesions on the follow-up brain CT scan were compared with the initial scan and if any progression had occurred, it was noted (14). If the patient underwent surgical evacuation of an intracranial lesion, any un-evacuated hematomas present were assessed for progression. Data including Glasgow Coma Scale (GCS), ISS, and outcomes were collected prospectively in the Karolinska Traumatic Injury database. Additional parameters were collected retrospectively through the electronic medical record system TakeCare® (CompuGroup Medical Sweden AB, Farsta, Sweden),

and any missing was be interpreted as not performed as there is no loss of data within the system.

Outcome

Glasgow Outcome Scale (GOS) (21) was evaluated at approximately 12 months following the trauma by questionnaire concerning functional outcome. GOS is classified as a categorical variable, where GOS 1 indicates death, GOS 2 vegetative state, GOS 3 severe dependent state, GOS 4 moderate independent state, and GOS 5 full recovery. Dichotomized GOS comprises the two categories GOS 1–3 (unfavorable) and GOS 4–5 (favorable) outcome.

MEA Measurements

The Multiplate® (Roche Diagnostics, Basel, Switzerland) unit was used to assess MEA values. This is an impedance aggregometry method (22) where multiple electrodes are immersed in a blood sample. Upon initial contact with blood, platelets coat the electrodes. Following stimulation with platelet agonists, there is a large increment in platelet aggregation upon the electrodes, quantified as increased impedance (23), and measured as an area under the curve (AU), providing the measurement unit. The method has been previously used in settings comprising intracranial pathology (24).

Using MEA, we evaluated three different platelet activation pathways: the arachidonic acid receptor (ASPI), $P2Y_{12}$ receptor (ADP), and thrombin receptor (TRAP). These receptors are specifically inhibited by the pharmacological compounds: cyclooxygenase (COX) inhibitors (ASPI), $P2Y_{12}$ inhibitors (ADP), and glycoprotein IIb/IIIa antagonists (TRAP) (23). Indications for MEA tests were as follows: antiplatelet therapy; heavy perioperative bleeding judged by the surgeon and/or intensivist; clinical bleeding propensity; or abnormal baseline hematologic values. MEA tests were occasionally taken solely at the initiative of a clinician. Commonly, if a first sample was obtained and particularly, if it guided treatment, the initial measurement was followed by subsequent measurements to evaluate treatment effect. Currently, there are no internationally established guidelines advising when MEA samples should be acquired in NICU TBI patients. In this study, up to nine MEA measurements were recorded per patient. The "first value" was defined as the value in closest proximity to admission. Two different MEA devices (both Multiplate®) were used. The reference intervals were similar for the two devices, with the exception of ASPI, which was 71–115 AU in a local NICU apparatus and 65–119 AU in the central laboratory apparatus. The reference interval for the local apparatus was used in further analyses.

Statistical Analysis

The full statistical protocol is described in the Supplementary Materials and Methods in the Supplementary Material. Demographic data were presented as mean ± SD, median (interquartile range), or count (%). Spearman correlation (rho, ρ) was used to assess correlation between different platelet receptor values, in addition to visual scatterplots. The distribution of MEA was examined using the Shapiro–Wilk test, and inferential analysis was conducted using the Mann–Whitney *U* test and the Wilcoxon signed rank test where appropriate. We assessed longitudinal changes of MEA values (ASPI) using linear mixed effect models with random and fixed effects in the lme4 package (25) in R. Model criteria were evaluated graphically and deemed to be fulfilled. The compiled model was obtained using likelihood ratio tests, where after the model with the lowest Akaike Information Criterion value was chosen. Various outcome prediction models were used. First, to assist hypothesis generation, outcome was visualized using recursive partitioning decision trees in the R package rpart (26) and rattle (27) with GOS as dependent variable (21). Independent variables were those previously shown to be the major predictors for TBI outcome [International Mission for Prognosis and Analysis of Clinical Trials in TBI (IMPACT) variables] (28), combined with coagulation tests clinically available at our clinic, including the first MEA values. Regression models were used to assess association with outcome (GOS, proportional odds) and hemorrhagic progression (logistical). The univariate analysis on outcome was performed with unimputed data. Variables included in univariate analysis were chosen with guidance from Fabbri and colleagues (7), or if hypothesized to modulate platelet function or coagulation. Variables emanating significant in univariate analysis were analyzed in a step-up followed by a step-down model, ultimately resulting in the final multivariable model using the R package rms (29). The statistical software program Rstudio® (R Foundation for Statistical Computing, Vienna, Austria; http://www.R-project.org) was used in all calculations.

Missing Data

Missing values relevant for outcome analysis were plotted (Figure S1 in Supplementary Material) using the R package neato (30). As several variables contained missing values to a large extent, we employed a standard multiple imputation approach using seven imputations of the dataset using the R package mice (31). The individual imputations and the pooled result from them were used in subsequent analyses. This approach has been used by the IMPACT study group (32) and is favored by the statistical literature (33).

RESULTS

Demographics

Between 2010 and 2014, 387 TBI patients were admitted to the NICU at the Karolinska University Hospital. Of these, 178 underwent MEA analysis and were included. Patient demographics are presented in **Tables 1** and **2**, respectively. Admission GCS varied from 3 to 15, with a median GCS of 7 (severe TBI). In total, 48% suffered an unfavorable outcome. At hospital admission, 24% of the patients had COX inhibitor treatment. The median MEA values for the first ASPI and ADP measurements were lower than the reference interval, meanwhile the first TRAP measurement was within it. Of all patients' first MEA values, 124 (70%) had pathologically low ASPI values, 109 patients (61%) had low ADP, and 65 patients (37%) low TRAP values. Throughout the hospital stay, 112 patients (63%) received a platelet transfusion, with a median total volume of 600 ml.

TABLE 1 | Patient demographics.

Variable	Type (unit)	Total number of patients: 178
Gender	Male/female	133/45 (75/25)
Age	(years)	54 (37–65)
Oxygen saturation SoA	(%)	96 (93–98)
	Missing	68 (38)
Blood pressure SoA	Systolic (mmHg)	130 (120–150)
	Missing	69 (39)
Admission GCS	GCS 3	53 (30)
	GCS 4–5	15 (8)
	GCS 6–8	31 (17)
	GCS 9–13	47 (26)
	GCS 14–15	32 (18)
	Median	7
Pupil responsiveness admission	Normal	138 (78)
	Unilateral unresponsive	18 (10)
	Bilateral unresponsive	19 (11)
	Missing	3 (1.7)
Extracranial injury (multitrauma)	Present	46 (26)
Radiological findings	Midline shift (mm)	3 (0–9)
	Progression hematoma	60 (34)
	Epidural hematoma	20 (11)
	Dual subdural hematoma	11 (6)
	Intraventricular bleeding	28 (16)
	Subarachnoid hemorrhage basal cisterns	42 (24)
	Subarachnoid hemorrhage convexity	121 (68)
Stockholm CT score	Total score	22 ± 9.6
Final GOS	GOS 1 (dead)	22 (12)
	GOS 2 (vegetative)	2 (1)
	GOS 3 (severe, dependent)	61 (34)
	GOS 4 (moderate, independent)	56 (31)
	GOS 5 (recovered)	37 (21)
	GOS 1–3 (unfavorable)	85 (48)
	GOS 4–5 (favorable)	93 (52)

In total, 178 patients were included. Data are depicted as count (%) if categorical and otherwise as median (interquartile range) or mean ± SD, if continuous. If there were any missing values, they are denoted as count (%) after each variable of concern.
GCS, Glasgow Coma Scale; GOS, Glasgow Outcome Scale; SoA, scene of accident.

TABLE 2 | Coagulation status.

Variable		Patient values	Reference interval (unit)
Platelet count admission		212 (175–248)	145–348 (10^9/l)
APTT admission		31 (29–36)	28–40 (s)
	Missing	9 (5)	
INR admission		1.1 (1–1.2)	<1.2 (INR)
	Missing	6 (3)	
ASPI, first value		51 (23.5–79)	71–115; 65–119 (AU)
ADP, first value		51 (31–69)	57–113; 57–113 (AU)
TRAP, first value		95 ± 35.6	84–128; 84–128 (AU)
	Missing	1 (0.56)	
Platelet transfusion	Transfused	112 (63)	
	Missing	4 (2)	
Platelet transfusion dose	Volume	600 (0–1,142)	(ml)
	Missing	10 (5.6)	
Erythrocyte transfusion	Transfused	102 (57)	
Fresh frozen plasma transfusion	Transfused	80 (45)	
COX inhibitor treatment	Treatment before admission	42 (24)	
ADP inhibitor treatment	Treatment before admission	5 (2.8)	

In total, 178 patients were included. Data are depicted as count (%) if categorical and otherwise as median (interquartile range), or mean ± SD, if continuous. When applicable, reference intervals are reported as those that at the time point of the study were used at the Karolinska University Hospital. Measurement units are given in parentheses next to the reference interval value. MEA measurements were obtained using two different devices, which had slightly different reference intervals, both of which are reported.
ADP, $P2Y_{12}$ receptor; APTT, activated partial thromboplastin time; ASPI, arachidonic acid receptor; AU, area under the curve; COX, cyclooxygenase; INR, international normalized ratio; TRAP, thrombin receptor; MEA, multiple electrode aggregometry.

Correlation between Different MEA Variables

There was a significant positive correlation between all three platelet receptors' first MEA values (Figures S2A1–C1 in Supplementary Material). For the ASPI versus TRAP receptor, $\rho = 0.59$ ($p < 0.001$). For the ASPI versus ADP receptor, $\rho = 0.71$ ($p < 0.001$). Finally, for the ADP versus the TRAP receptor, $\rho = 0.66$ ($p < 0.001$). Based on this, we pursued our examinations primarily using the ASPI receptor.

Platelet Modulating Pharmacological Compounds

The first MEA values were examined on 127 patients of which 30 had received COX inhibitor treatment. We excluded all patients who had received a platelet transfusion before MEA values were obtained. MEA values were lower for patients treated with COX inhibitors compared with those who were not (**Figure 1A**), $p < 0.001$. Notably, the median ASPI value was below the lower reference interval, independent of COX inhibitor therapy. In total, ~66% of patients without COX inhibitor treatment demonstrated ASPI values below the lower reference interval. MEA values increased following a platelet transfusion (**Figure 1B**), $p < 0.001$.

Longitudinal Pattern

Immediately following TBI, platelet function (ASPI) was low and thereafter increased over time (**Figure 2A**; **Table 3**, $p < 0.001$). The same graphical trend was seen when excluding patients on COX inhibitors (Figure S3B in Supplementary Material), or that had been transfused with platelets (Figure S3C in Supplementary Material). Moreover, platelet count was positively correlated with MEA values (Figure S4 in Supplementary Material; **Table 3**, $p < 0.001$). Patients presenting with ongoing COX inhibitor treatment had consistently lower ASPI values than patients not treated with COX inhibitors (**Figure 2A**; **Table 3**, $p = 0.026$), except for one time point, which, however, was preceded by an increase in the number of platelet transfusions (**Figure 2B**).

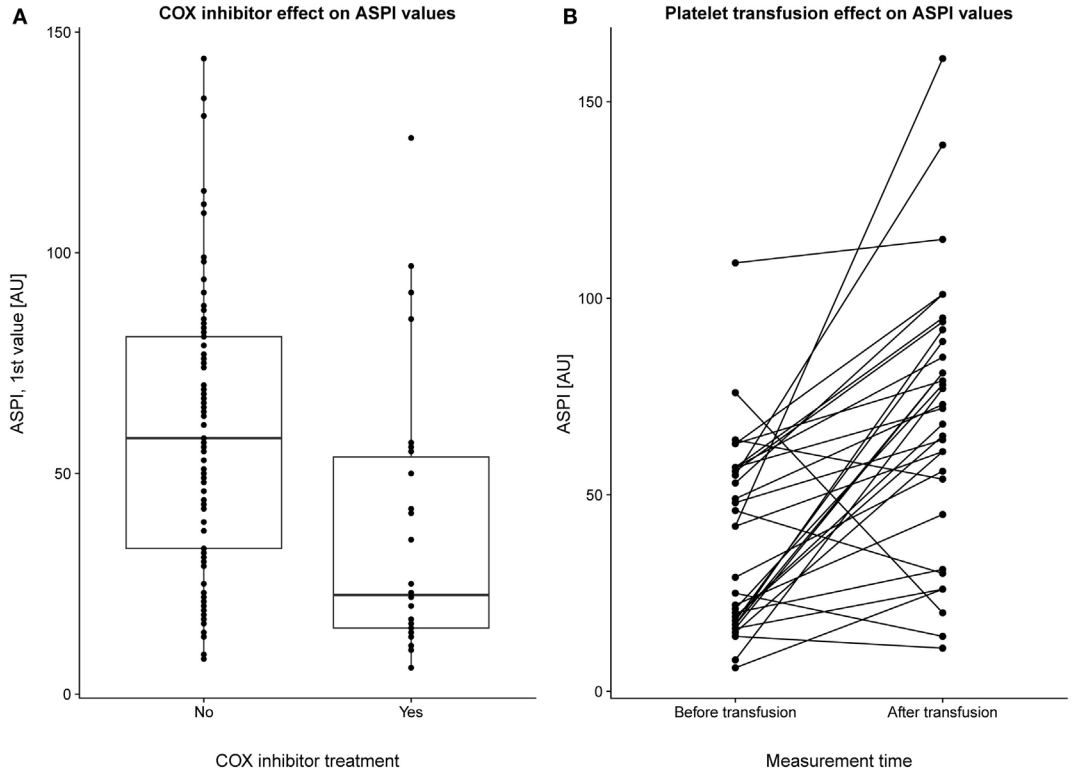

FIGURE 1 | Pharmacologic modulation of multiple electrode aggregometry (MEA) values. The first arachidonic acid receptor (ASPI) values (indicated as individual data points and as summarizing boxplots) for a subset of patients with and without cyclooxygenase (COX) inhibitor treatment (and without preceding platelet transfusions) are depicted **(A)**. Overall, COX inhibitor treatment yielded lower ASPI results, $p < 0.001$. In panel **(B)**, a subgroup of patients who had undergone MEA measurements before and after transfusion were selected. Individuals are plotted with their respective ASPI value at the first and second measurement, with a line connecting each individual. No account was taken for how long time that had passed between the first and second MEA measurement, but there was still a strongly significant ($p < 0.001$) increase in platelet function following transfusion.

MEA As Predictor of Hemorrhagic Progression and Outcome

Outcome Illustration Using Decision Trees

The decision tree gives a graphic representation of information content that could aid rule based interpretation of the data. Decision trees from all seven imputations were compared visually and showed congruency overall (data not shown), of which one representative is shown (**Figure 3**). Low first ASPI values appear to distinguish worse outcome in patients <68 years with higher GCS (≥5.5) scores at admission. Although already on level three of the decision tree, this degree of subgrouping must be interpreted with care and as hypothesis generating.

Regression Analyses of MEA versus Outcome and Hemorrhagic Progression

Relations of MEA values toward GOS levels were visualized with conditional density plots (CD plots, **Figures 4A–C**). ASPI values had a u-shaped relation to GOS (**Figure 4A**), where both high and low levels were associated with a more unfavorable outcome. Although areas with limited data must be interpreted with caution, the CD plots can help visualize the information content, and accuracy of univariate regression will be related to the slope of the lines discriminating levels in areas with most data. ADP showed a similar tendency as ASPI, but fluctuations probably reflecting "noise," make interpretation difficult (**Figure 4B**). Higher TRAP values were visually associated with favorable GOS (**Figure 4C**). The results of the univariate analysis (**Table 4**) were congruent with the CD plots, with ASPI ($p = 0.0389$) and TRAP ($p = 0.0086$), but not ADP being significantly related to GOS. The IMPACT variables (28) that form the "base model" [age, admission GCS, pupil responsiveness, Stockholm CT score, oxygen saturation, and blood pressure (BP)] were significant in univariate analysis with the exception of BP. Additional significant variables in univariate analysis were the coagulation predictors APTT ($p = 0.0020$), COX inhibitor treatment ($p = 0.0176$), platelet transfusion ($p = 0.0030$), and intracranial radiologic hemorrhagic progression ($p = 0.0001$). The ISS was not significantly related to outcome. Following step-up (Table S1 in Supplementary Material) and step-down models, radiologic intracranial hemorrhagic progression was the only significant variable aside from the base model in the multivariable model (**Table 5**). Overall, the Nagelkerke's pseudo-R^2 had a mean of 39.8% of the pseudo- explained variance of outcome prediction, for the seven different imputations. Since elderly patients are more likely to have COX inhibitors, the age variable in IMPACT

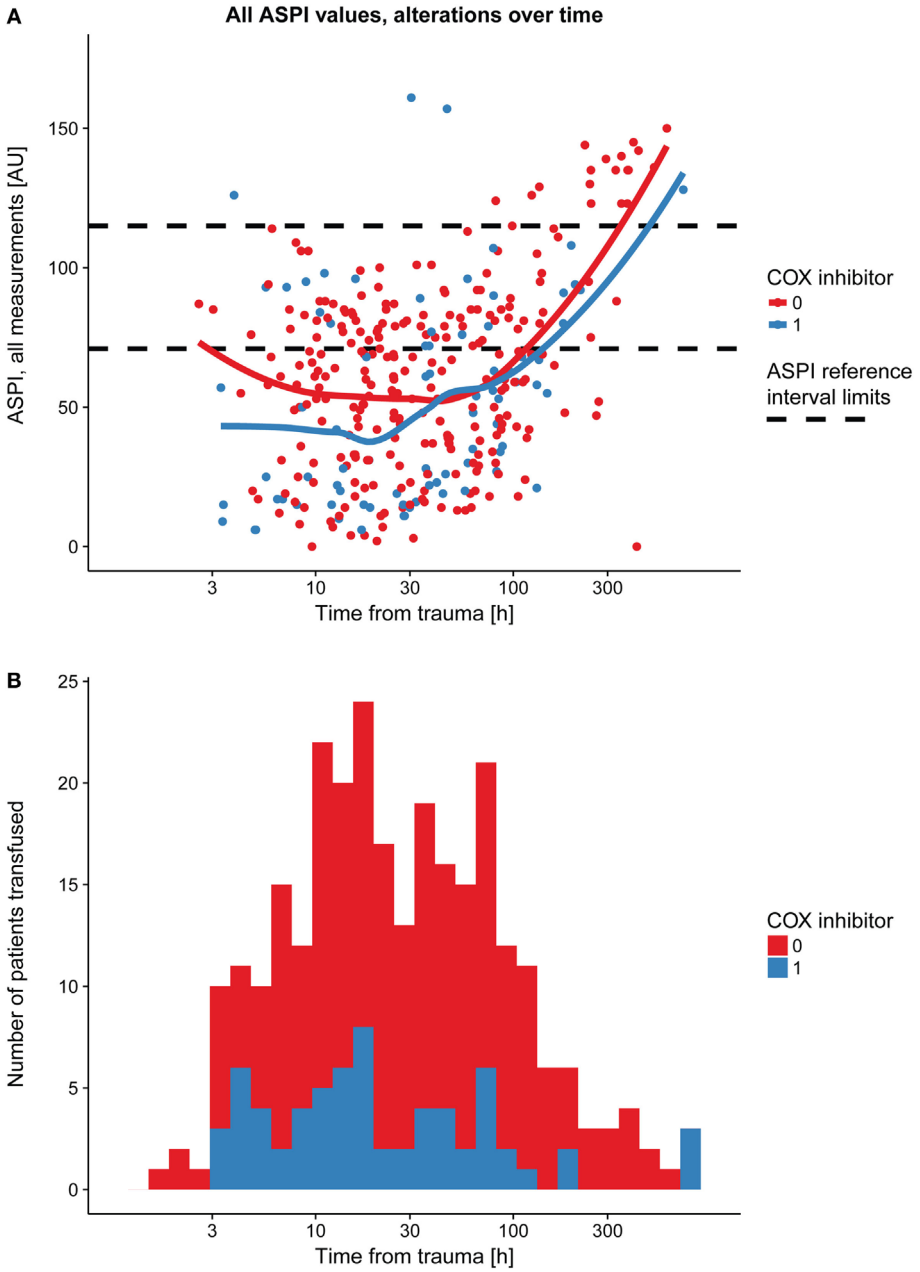

FIGURE 2 | Platelet function alterations over time following TBI, dependent on prehospital cyclooxygenase (COX) inhibitor treatment. Patients with COX inhibitor treatment before hospital admission demonstrated a consistently lower arachidonic acid receptor (ASPI) value compared with those without COX inhibitor treatment **(A)**, as showed by the two LOWESS curves. The black dashed line denotes the ASPI reference interval (71–115 AU). At one time point, however, there was a partial overlap, which, as shown in panel **(B)**, might be explained by an increase in platelet transfusions at the time point preceding the overlapping LOWESS curves in panel **(A)**. Abbreviations: AU, area under the curve; LOWESS, locally weighted scatterplot smoother; TBI, traumatic brain injury.

might affect the interpretation of COX inhibitors. Omitting age from the base model resulted in COX inhibitors being a significant predictor of worse outcome in the multivariable model (data not shown).

Relations of MEA as predictors toward hemorrhagic progression were visualized with CD plots (**Figures 4D–F**) suggesting ASPI values to have a u-shaped association with an increased percentage of hemorrhagic progression (**Figure 4D**), similar to GOS. However, this was not significant in univariate analysis (Table S2 in Supplementary Material). APTT was significant in univariable analysis ($p = 0.0178$, Nagelkerke's pseudo-$R^2 = 0.058$, Table S2 in Supplementary Material). Admission GCS and Stockholm CT score were borderline significant in univariate analysis (Table S2 in Supplementary Material). Since no variables were significant except APTT, no step-down or multivariable analysis was conducted.

TABLE 3 | Linear mixed effect model for ASPI values among TBI patients.

Fixed effect variable	Estimate	SE	*p*Value (likelihood ratio test)
Time from trauma	0.10627	0.02	<0.001
Platelet count	0.09221	0.01381	<0.001
COX inhibitor	−9.47869	4.23748	0.02583

Random intercepts: p < 0.001 [p value generated using (34)].
AIC value final model: 3,265.7.
Variables affecting ASPI values were compared across measurement time points.
Variables that affected ASPI when examined graphically were included in the model.
p Values were obtained using the likelihood ratio test, or as otherwise reported.
Included random effects were random intercepts (subject ID), presented at the bottom of the table.
AIC, Akaike Information Criterion; ASPI, arachidonic acid receptor; COX, cyclooxygenase; TBI, traumatic brain injury.

DISCUSSION

To the best of our knowledge, this is the first study to investigate the clinical utility of a platelet function method in a clinical TBI setting. We found that

(i) Platelet function, as indicated by MEA, exhibits a temporal profile even in the absence of platelet inhibitors where MEA values are generally low initially, and subsequently increase over the days following TBI. If this also reflects a clinically significant coagulopathy is yet unknown.
(ii) The first ASPI and TRAP values were associated with long-term outcome in univariable analyses, albeit they did not contribute with any independent information when adjusting with known outcome predictors.
(iii) Radiologic intracranial hemorrhagic progression, an important predictor of long-term outcome, could not be predicted using MEA values.
(iv) MEA may in part be able to identify patients who arrive with TBI using COX inhibitors from a general coagulopathy.

Trauma-induced coagulopathy, one of the secondary injuries following TBI, is a poorly defined condition incorporating a hypo- and a hypercoagulable state (2). The pathophysiology is considered distinct from coagulopathy following other types of trauma, due to brain-specific features, e.g., high tissue factor levels, interaction between the disrupted blood–brain barrier/plasma proteins, and microparticle influence (35, 36). New methods to diagnose TIC have gained clinical interest, in particular platelet dysfunction, observed following TBI in both studies of animal models (37, 38) and human subjects without platelet inhibitory treatment (13, 38–41).

Platelet receptor values have but rarely (39) been characterized longitudinally following TBI. Numerous studies claim that trauma itself induces coagulopathy [reviewed in Ref. (35)]. Here, we saw that ~66% of the patients who had not received any COX inhibitor treatment exhibited pathologically low ASPI values upon admission, as defined by current reference levels. This finding is consistent with previous studies on coagulopathy prevalence in severe TBI (2). We show a longitudinal alteration of MEA values, with initially low values, followed by a later increase. This is consistent with a previous study (39), however, we measured over a longer time period and quantified the relationship longitudinally. The increase in MEA values was also observed in the absence of platelet transfusions, implying that this is a biological phenomenon reflecting a trauma-induced alteration of MEA values. This finding highlights the need for future studies to investigate MEA relations to clinically significant bleeding in TBI patients, even in the absence of platelet inhibitors. In addition, cutoff values and cutoffs related to elapsed time need to be established. In summary, this is the first quantitative examination of how MEA values alter following TBI.

In outcome analysis, CD plots of GOS and intracranial hemorrhagic progression exhibited a similar u-shaped relation between ASPI/GOS and ASPI/intracranial hemorrhagic progression, with both high and low levels suggesting worse outcomes. This implies that there might be ASPI values that are optimal to evade hemorrhagic progression and accordingly, affect GOS. However, these data should be interpreted cautiously, as the CD plots contained few observations at very low and high levels of ASPI. In univariate analysis (dependent variable GOS), both the first ASPI and TRAP values were significant. In multivariable analysis, this effect was no longer seen, meaning that ASPI and TRAP are meaningful for outcome prediction *per se*, but that they do not provide independent information when one has access to other base line variables. An interpretation of this is that pathological ASPI and TRAP values reflect a significant coagulopathy that could be related to deterioration and thus lower GCS. Prospective and clinician-blinded studies will be needed to confirm this. Previous studies show conflicting results. In one study, the TRAP, and ASPI receptors were independent predictors of mortality (39). In another study, unfortunately unadjusted for confounders, ADP and TRAP values were different between survivors and non-survivors in a mixed trauma population (13). Yet others have found ADP, but not ASPI, to be a predictor of mortality (41). Finally, there are also studies without relation between platelet function results and outcome, hemorrhagic progression, or mortality (42). The lack of congruency between these studies, including ours, indicates that there is a pressing need for future prospective studies on patients with severe TBI.

Radiologic intracranial hemorrhagic progression was a strong predictor of GOS. However, in univariate analysis of predictors for radiologic hemorrhagic progression, APTT at admission was the only significant variable. GCS and Stockholm CT score at admission were borderline significant, implying that a worse initial clinical status was associated with future progression and deterioration. The finding of APTT is interesting, but not the focus of this study.

Platelet inhibitors are common among TBI patients due to demographic alterations (5, 43). We found consistently lower ASPI values among patients on COX inhibitor treatment, congruent with others (39, 43). As has been similarly shown (43), the median ASPI was below the reference interval also for patients without known COX inhibitor treatment, making it difficult to distinguish pharmacologic from trauma-induced platelet receptor hypofunction. In outcome analysis COX inhibitor treatment was significantly related to GOS in univariate analysis, but not against radiologic intracranial hemorrhagic progression. In multivariable analysis, COX inhibitor treatment was no longer

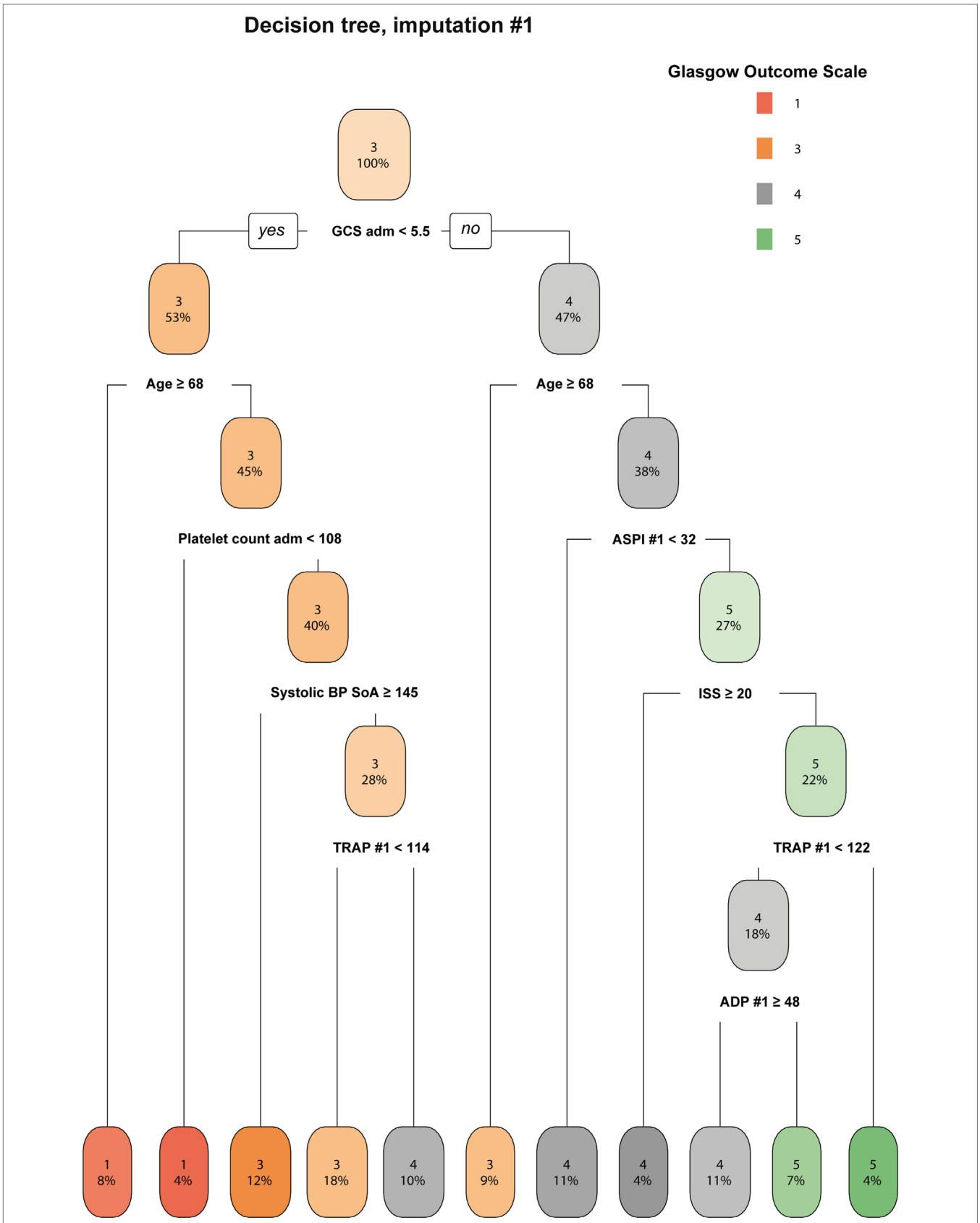

FIGURE 3 | Continued

FIGURE 3 | A decision tree for determining the importance of ASPI. A representative imputation of one of the decision trees is depicted. Each node in the tree denotes the predicted GOS value (indexed by color and GOS category number), followed by the percentage of patients belonging in each node. While moving downwards in the tree, GOS changes depending on the independent variables and the percentages of patients who pertain to the stipulated criteria decrease accordingly. Consistent across all imputations, ASPI values in the range of 32–68 affected the determination of Glasgow Outcome Scale (GOS) 4–5 among younger patients with higher GCS (only one imputation shown). Radiologic score was defined as the Stockholm CT score (19). Data values are presented in the same units as can be found in **Tables 1** and **2**, respectively. Abbreviations: adm, admission; ADP, $P2Y_{12}$ receptor; ASPI, arachidonic acid receptor; BP, blood pressure; GCS, Glasgow Coma Scale; ISS, Injury Severity Score; SoA, scene of accident; TRAP, thrombin receptor; #, ordered number of sample; GOS, Glasgow Outcome Scale.

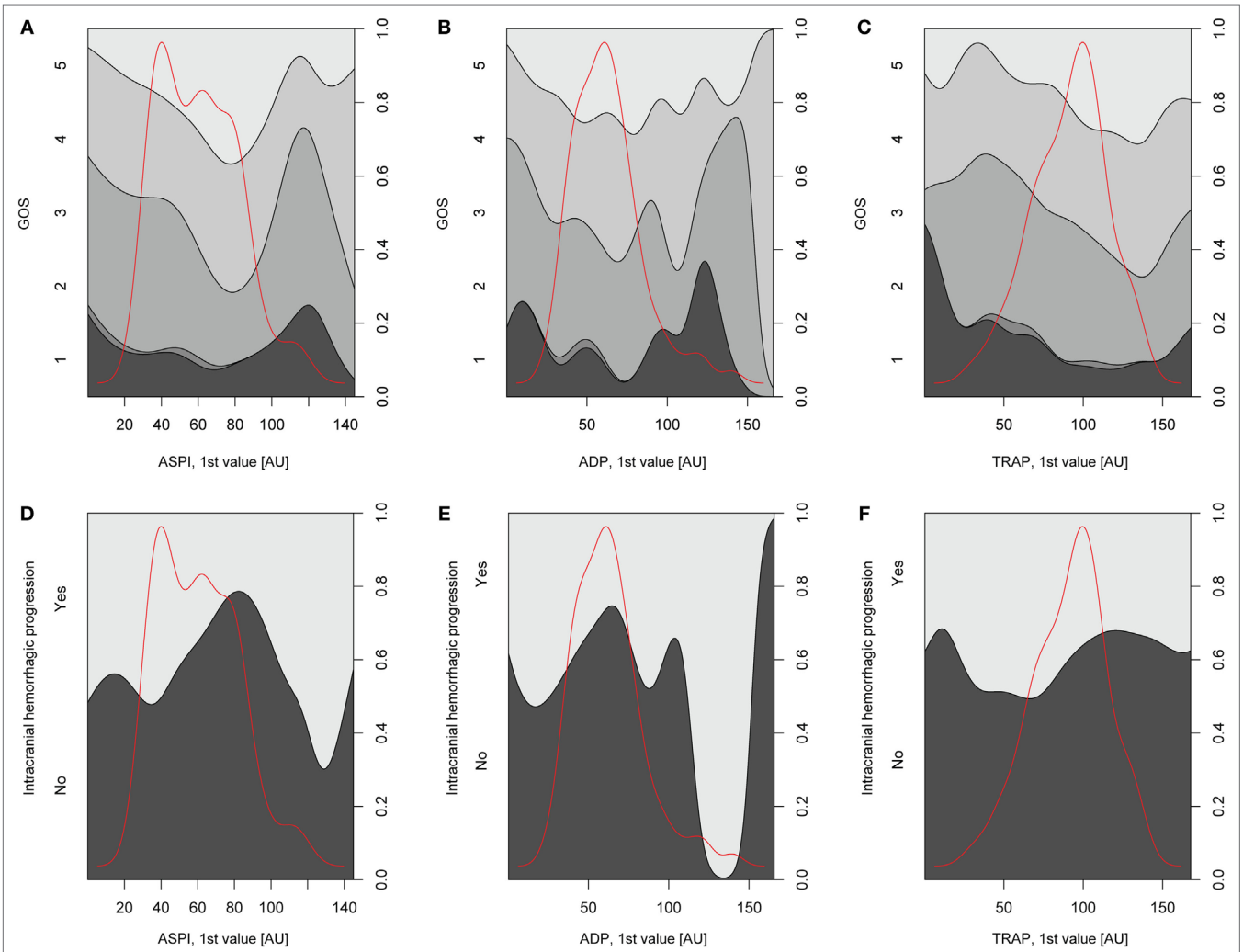

FIGURE 4 | Depiction of univariate analysis of selected variables hypothesized to influence Glasgow Outcome Scale (GOS) or hemorrhagic progression. In panels **(A–C)**, conditional density (CD) plots for the different platelet receptors versus GOS are demonstrated. In a CD plot, the left *y*-axis depicts the category of the dependent variable (in this case GOS). The right *y*-axis depicts outcome proportions for each value of the *x*-axis' independent variable (in this case the different platelet receptors). The red line overlaid in the plot depicts the amount of observations across different values of the independent variable and has therefore no relation with the *y*-axis. As the density plot demonstrates, there were fewer patients with extreme values of the independent variable, respectively, thus decreasing the reliability of the relationship between the independent variable and GOS at these values of the independent variable. In panel **(A)**, arachidonic acid receptor (ASPI) values generated high amounts of unfavorable outcome (GOS 1–3) at low and high ASPI values, respectively, although there was scarce amount of data for higher ASPI values. The same trend was not readily detectable for the $P2Y_{12}$ receptor (ADP) due to a fluctuating curve **(B)** but was evident for the TRAP **(C)**. In panels **(D–F)**, CD plots for the different platelet receptors versus hemorrhagic progression are depicted. A similar u-shaped relation as in panels **(A–C)** was detected for ASPI but now toward increased risk of hemorrhagic progression **(D)**. A similar trend was seen in **(E)** but not as readily in panel **(F)**, depicting the $P2Y_{12}$ (ADP) receptor and thrombin receptor (TRAP), respectively.

significant. COX inhibitor treatment has previously been shown to predict worsening lesions on follow-up radiology (7) and to predict unfavorable outcome [reviewed in Ref. (11)]. Other studies have failed to demonstrate this (42). As the majority of patients on COX inhibitor treatment are older, when adjusting for age, the true effect of the treatment might disappear. When we

TABLE 4 | Univariate analysis of variables and correlations to final GOS.

Independent variable	*p*Value	Pseudo-R^2
Base model		
Age	0.0008	0.067
GCS at admission	<0.0001	0.167
Pupil responsiveness	0.0070, <0.0001	0.130
Stockholm CT score	<0.0001	0.208
Oxygen saturation at SoA	0.0282	0.044
Blood pressure at SoA	0.1401	NS
Additional variables assessed		
Injury Severity Score	0.4317	NS
ASPI, 1st value	0.0389	0.026
ADP, 1st value	0.2874	NS
TRAP, 1st value	0.0086	0.042
TPK at admission	0.3616	NS
INR at admission	0.4198	NS
APTT at admission	0.0020	0.060
Platelet transfusion	0.0030	0.054
COX inhibitor treatment	0.0176	0.034
Radiologic intracranial hemorrhagic progression	0.0001	0.104

Results from univariate analysis of independent variables assumed to affect Glasgow Outcome Scale (GOS) are depicted. Apart from the IMPACT variables (28), these comprised platelet count, coagulation measurements, variables hypothesized to modulate platelet function, and radiologic intracranial hemorrhagic progression. Platelet transfusion, COX inhibitor treatment, and radiologic intracranial hemorrhagic progression were used as dichotomous variables.

ADP, $P2Y_{12}$ receptor; APTT, activated partial thromboplastin time; ASPI, arachidonic acid receptor; COX, cyclooxygenase; CT, computerized tomography; GCS, Glasgow Coma Scale; INR, international normalized ratio; SoA, scene of accident; TRAP, thrombin receptor.

TABLE 5 | Multivariable proportional odds analysis of variables affecting final GOS.

Independent variable	OR	CI	*p*Value
Age	0.966	0.948–0.984	0.000117
GCS admission	1.130	1.05–1.21	0.001612
Pupil responsiveness	0.596	0.355–1.00	0.0707
Stockholm CT score	0.945	0.914–0.977	0.00176
Oxygen saturation SoA	1.06	1.00–1.11	0.0685
Blood pressure SoA	1.00	0.992–1.01	0.409
Radiological intracranial hemorrhagic progression	0.363	0.196–0.671	0.00479

Mean Nagelkerke's pseudo-R^2 0.398 = 39.8%

Results from multivariable analysis of independent variables assumed to affect Glasgow Outcome Scale (GOS) are depicted. Data are presented as odds ratio (OR) and 95% confidence interval (CI) for one imputation. p Values are reported as the pooled p value from all imputations (n = 7).

CT, computerized tomography; GCS, Glasgow Coma Scale; SoA, scene of accident.

conducted the analysis omitting age adjustment, COX inhibitor treatment was a predictor of worse outcome, leading us to believe that not only could this account for previous discrepancies, but also, that until convincingly shown in a prospective randomized material, COX inhibitor treatment should be considered a risk factor for worse prognosis.

A theoretical treatment for platelet dysfunction is platelet transfusion. Based on ASPI levels, we distinguished an increased platelet function following transfusion, as expected (8). In outcome analysis, platelet transfusion was as a predictor of worse outcome in univariate, but not multivariable, analysis. These results should be interpreted very cautiously, since a group more heavily transfused often is one with more extensive injuries, and patients diagnosed with a hemorrhagic progression might have received transfusions to a larger extent than those who were not. Therefore, we cannot claim that transfusions are inadvisable. Yet, the implementation of MEA has resulted in diagnosis of presumptive pathology, for which there is no widely acknowledged treatment regimen (39). A review of five retrospective TBI studies where patients had antiplatelet therapy before admission found both beneficial and harmful effects of platelet transfusion therapy (44), whereas platelet transfusion was not significantly associated with outcome in later studies (45). In conclusion, the clinical value of platelet transfusions in the treatment of TIC therefore remains to be convincingly shown and especially in the setting of pathological MEA values in patients not receiving platelet inhibitors. Notably, the AABB (formerly the American Association of Blood Banks) could not recommend either for or against platelet transfusion for the subgroup of patients on antiplatelet therapy and traumatic intracranial hemorrhage (46).

This study should be pursued by a blinded randomized prospective multicenter study on isolated (non-multitrauma) TBI patients using GOS and lesion progression as outcome variables. MEA measurements should be taken at fixed, consecutive time points. Using these data, it would be possible to establish eligible reference intervals at different time points and conduct outcome analysis.

Limitations

This study holds all the limitations of a retrospective study. Residual confounding, confounding by indication, and treatment bias must be assumed to exist in our data. These highlight problems with retrospective observational analyses and emphasize the need for blinded prospective trials. However, in lack of such MEA is often performed as a screening to identify if unconscious TBI patients are on platelet inhibitors. This will present the physician with a multitude of pathological MEA values in patients without platelet inhibitors, and without a metric for interpretation. This highly motivates an observational study such as this, despite its retrospective nature. Moreover, despite caveats, this is one of the first and larger studies to analyze a large cohort of mild-to-severe TBI patients and as we have had access to large amounts of prospectively collected data, we believe our data set is clinically valid for an NICU TBI population. Further, we have access to some unique variables like the trauma time, successfully registered in all prehospital records. Importantly, we have used this for a reliable characterization of platelet function longitudinally, which can rarely be done in equally large materials elsewhere. Still, MEA samples were to a large extent obtained at different time points, and treatments given at others. Moreover, our assessment of intracranial hemorrhage progression could be a bit sensitive as any lesion present was taken into account. Previous studies have defined set threshold, such as a minimal blood volume of 2 ml on the first CT scan (47). Further, we did not account for concomitant infection or disseminated intravascular coagulation (DIC) (48), both of which, but in particular DIC (48–50) could have confounded our data. This is complicated by the fact that it is difficult to distinguish between different types of coagulopathy

following TBI (50). Altogether, we have been able to statistically compensate for some of the lack of standardization in our heterogeneous data set. Yet, the nature of our data and study design limits the extent to which conclusions can be drawn from this cohort, and our findings should at this point be seen as hypothesis generating. In aggregate, this study represents a large data set from a clinical setting that many physicians will be presented with and that will require decisions. This highly motivates this retrospective hypothesis generating study in wait of blinded prospective studies.

CONCLUSION

We present the first larger investigation of the clinical utility of platelet function measurements using MEA in NICU treated TBI patients. Following TBI, a general longitudinal trend, with initially low MEA values and a subsequent increase over days is seen, indicating a pathophysiological link. MEA levels were affected by both COX inhibitor treatment and platelet transfusion. Progression of intracranial hemorrhage is an important predictor of poor TBI prognosis but MEA values could not significantly predict this condition. Both ASPI and TRAP values were associated with outcome, but did not add any independent information in presence of other outcome predictors. In summary, these findings warrant further prospective, blinded trials to full understand the utility of MEA in TBI patients.

ETHICS STATEMENT

The study was carried out in accordance with Swedish legislation, the Declaration of Helsinki, and the specific recommendations stipulated by the local ethics committee in Stockholm County, Centrala Etikprövningsnämnden (the Central Ethical Review Board). The study was exempt from written informed consent, as it was carried out retrospectively on data base material, was purely observational, and did not inflict on patient treatment. The local ethics committee (the Central Ethical Review Board) approved the protocol of this study (diary numbers 2014/1488-31/5 and 2015/1675-31/1).

AUTHOR CONTRIBUTIONS

CL, ET, AF, MN, DN, B-MB, and MS designed and planned the study. CL, ET, and MN acquisitioned the data. CL, ET, AF, and DN analyzed and interpreted the data. CL drafted the manuscript. CL, ET, MN, AF, DN, MS, and B-MB revised the manuscript critically. All the authors read and approved the final manuscript and agreed to be accountable for all aspects of the work.

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SUPPLEMENTARY MATERIAL

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REVIEW
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The Neurological Wake-up Test—A Role in Neurocritical Care Monitoring of Traumatic Brain Injury Patients?

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The most fundamental clinical monitoring tool in traumatic brain injury (TBI) patients is the repeated clinical examination. In the severe TBI patient treated by continuous sedation in a neurocritical care (NCC) unit, sedation interruption is required to enable a clinical evaluation (named the neurological wake-up test; NWT) assessing the level of consciousness, pupillary diameter and reactivity to light, and presence of focal neurological deficits. There is a basic conflict regarding the NWT in the NCC setting; can the clinical information obtained by the NWT justify the risk of inducing a stress response in a severe TBI patient? Furthermore, in the presence of advanced multimodal monitoring and neuroimaging, is the NWT necessary to identify important clinical alterations? In studies of severe TBI patients, the NWT was consistently shown to induce a stress reaction including brief increases in intracranial pressure (ICP) and changes in cerebral perfusion pressure (CPP). However, it has not been established whether these short-lived ICP and CPP changes are detrimental to the injured brain. Daily interruption of sedation is associated with a reduced ventilator time, shorter hospital stay and reduced mortality in many studies of general intensive care unit patients, although such clinical benefits have not been firmly established in TBI. To date, there is no consensus on the use of the NWT among NCC units and systematic studies are scarce. Thus, additional studies evaluating the role of the NWT in clinical decision-making are needed. Multimodal NCC monitoring may be an adjunct in assessing in which TBI patients the NWT can be safely performed. At present, the NWT remains the golden standard for clinical monitoring and detection of neurological changes in NCC and could be considered in TBI patients with stable baseline ICP and CPP readings. The focus of the present review is an overview of the existing literature on the role of the NWT as a clinical monitoring tool for severe TBI patients.

Keywords: traumatic brain injury, neurocritial care, wake-up test, monitoring, stress response

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INTRODUCTION

Intense clinical monitoring is an integral part of the management or traumatic brain injury (TBI) patients. Neurological worsening, commonly defined as a decrease of two or more points on the motor component of the Glasgow Coma Scale (GCS-M) score, may occur rapidly and is associated with a poor outcome in TBI. Clinical deterioration may be caused by, e.g., ongoing hemorrhage

or increased brain swelling (1, 2) stressing the importance of repeated clinical evaluations. In addition, information from these neurological examinations may prove important in clinical decision-making, and lead to improved patient outcome. A swift neurological examination in the emergency setting can assess the gross structural integrity of the nervous system, enable an assessment of the injury severity and provide a prognostication tool for TBI. The components of the neurological examination used for monitoring can vary depending on the clinical situation, although assessment of the level of consciousness, neurological motor function, and assessment of pupillary size and reactivity is a minimal requirement.

The importance of repeated neurological evaluations was highlighted in the 1970s, when a large number of TBI patients were identified who on admission to the hospital were awake and able to talk but later died. The entity "talk-and-die" was coined (3), describing individuals in whom the severity of the initial, primary injury was insufficient to explain the poor outcome and that the occurrence of secondary, and presumably preventable and "avoidable," factors resulted in the fatal exacerbation of the disease. These findings prompted increased awareness and improved organization of TBI care, aided by the standardization of the neurological assessment through the introduction of the Glasgow Coma Scale (GCS) score in 1974 (4). Since TBI is a markedly heterogeneous disease that commonly shows an unpredictable and dynamic clinical course, adherence to protocols for neurological surveillance including repeated clinical evaluations of TBI patients is mandatory. Stricter guidelines and management protocols have presumably contributed to gradually reduced case fatality rates (5, 6), acknowledging that virtually all forms of TBI carry an inherent risk of exacerbation over time. The risk factors for neurological worsening in the mild–moderate TBI population have been addressed in numerous previous publications and guidelines (7–10), aiding the emergency room physician in the often difficult decision whether to perform neuroradiological investigations, admit for clinical monitoring or discharge the patient.

The use of prehospital sedation, paralysis, and intubation frequently used at the scene of the accident (11, 12) makes an assessment of neurological status of the TBI patient difficult. Following initial resuscitation of severe TBI patients, a neurological examination to obtain a post-resuscitation GCS score is recommended (13, 14) for TBI severity grading and for clinical decisions (15). Most severe TBI patients, after radiological investigations and surgical evacuation of space-occupying mass lesions, will also require continued care in a neurocritical care (NCC) unit. The NCC foundations for managing severe TBI consist of controlled ventilation, stress reduction using, e.g., continuous sedation, neuroimaging, and multimodality monitoring including the measurement of intracranial pressure (ICP) and cerebral perfusion pressure (CPP). Importantly, up to 40% of TBI patients show a clinically relevant neurological worsening within the first 48 NCC hours (16–18), arguing for repeated neurological examinations also during NCC.

Clinical examinations in NCC for severe TBI are controversial since they pose a dilemma—while sedation interruption is needed for the important neurological evaluation, an undesired stress response is commonly elicited. Is clinical monitoring using neurological assessments justified in modern NCC, and does the obtained information of the evaluation outweigh its potential risks? Furthermore, in the era of multimodality monitoring, what additional information is provided by neurological examinations and do they lead to changed management of the severe TBI patients? In the present overview, the rather scarce literature on neurological evaluation [here named the neurological wake-up test, NWT (19)], used as a clinical monitoring tool for severe TBI patients is discussed. Although the term "wake-up test" is used, it should be remembered that the response to interruption of sedation should not be regarded as an "awakening"; instead the response may be more comparable to an arousal reaction (20). Several terms to describe interruption of sedation strategies during NCC and/or the general intensive care units (ICUs) are used interchangeably in the medical literature, and may include protocols with our without concomitant evaluation of the neurological status. Conversely, sedation interruption or sedation lightening allowing for spontaneous breathing with the aim of speeding ventilator weaning is used in some protocols without simultaneously performing an NWT. Spontaneous awakening trials (SATs), spontaneous breathing trials (SBTs), daily interruption of sedation (DIS or IS-) trials, and lightening of sedation are examples of the used terminology. For the purpose of this review, medical databases (Medline, Scopus, and PsychINFO) were searched using the terms TBI, or any combination of brain or head trauma/injury, together with wake-up test, SBT, SAT, and/or lightening/interruption of sedation.

CONTINUOUS SEDATION AND SEDATION INTERRUPTION IN NCC AND GENERAL INTENSIVE CARE

Continuous sedation is used in general ICUs to prevent pain and anxiety, control agitation, and minimize patient discomfort, as well as to enable endotracheal tube tolerance needed for controlled mechanical ventilation (21–23). Additional NCC-specific aims of continuous sedation include the prevention of stress-related secondary insults, the reduction of cerebral energy metabolism and oxygen consumption, and seizure, ICP and temperature control (24). There are several sedatives for use in the NCC, the choice and combination of which may influence ICP and CPP control as well as cardiovascular stability. The most commonly used sedatives in NCC are arguably propofol and midazolam although compounds such as the selective α2-adrenergic agonist dexmedetomidine (25, 26) or the *N*-methyl-D-aspartate receptor antagonist ketamine (23) are more recent additions to the sedation armamentarium. The selected sedative, due to its plasma half-life and/or potential for lingering central nervous system effects, obviously influences the possibility of using the NWT in NCC monitoring.

Thus, continuous sedation is an integral part of both general ICU and NCC treatment protocols. However, this strategy is not without adverse effects since excessive doses of sedatives may lead to significant morbidity (27–29). Continuous sedation was also repeatedly shown to increase the incidence of ventilator-associated pneumonias, prolong mechanical ventilation, and result in

a higher mortality in ICUs (27, 29–31). Since delayed weaning from mechanical ventilation increases the risk of infections, it is desirable to reduced ventilator time (30, 32). These observations led to the implementation of daily interruption of continuous sedation (DIS) trials in general ICU (27, 29–31). A DIS protocol in combination with SBTs reduced the duration of mechanical ventilation and length of stay in general ICU, without increasing the complication frequency (33) or impairing long-term cognitive, psychological, and functional outcomes (27, 31, 34, 35). These data implied that DIS is beneficial in general ICU, although some uncertainty of patient safety and/or agitation has persisted and this strategy is used in only ca. 30–40% of ICU patients (27). However, in a study of Australian ICU patients, sedation interruption guidelines was not associated with a reduced duration of mechanical ventilation, and similar results were observed when a protocol-driven weaning protocol was evaluated (36). In a meta-analysis evaluating 699 critically ill ICU patients, DIS protocols were not found to reduce ventilator-associated pneumonias, duration of mechanical ventilation, length of ICU stay, or mortality although it did reduce the risk of tracheostomy (37). Finally, no strong evidence that DIS alters the duration of mechanical ventilation, mortality, length of ICU or hospital stay, adverse event rates, drug consumption, or quality of life was provided in a recent Cochrane review (32). In fact, an analgesia-delirium-sedation protocol, using carefully titrated sedation aimed to limit sedation depth and duration, was effective in reducing ventilator days and hospital stay in ICU patients without using DIS (38).

The current literature does not convey a clear message or substantial proof for benefit of minimizing sedation although the negative consequences of over-administration of sedatives are well established. Instead, protocol-driven control of sedation in combination with sedation scales and the use of sedatives with a short half-life may be equally effective to DIS for ICU patients (20). The level of evidence for minimizing complications by DIS in NCC is even lower. In a randomized control trial, a subgroup of TBI patients did not show significantly decreased ventilator time or ICU stay compared to controls when sedation was interrupted on a daily basis [see **Table 1**; (37)]. In contrast, the ability of sedation to reduce cerebral metabolic demand, ICP control, temperature management, and seizure control in NCC are undisputed.

At present, many controversies remain with regard to interruption of sedation in TBI patients where continuous sedation is part of the treatment strategy. At the current level of evidence, potential systemic benefits of the procedure derived from general ICU care may not be used as a key argument in favor of using NWTs in TBI care.

INDICATIONS FOR THE NWT IN NCC

Since there is no clear-cut evidence for a clinical benefit of sedation interruption in TBI patients, what other possible indications for the NWT are there? In particular, what additional information is sought by the NWT in the sedated and monitored TBI patient? The NWT is not mentioned in available TBI guidelines (13), and the use of the NWT may vary considerably among NCC centers. In our own Scandinavian survey, ca. 50% of NCC centers never used the NWT in daily routine care of TBI patients and there was a marked variation in the frequency of the NWTs in the remaining ones (44). Compared to midazolam, propofol sedation

TABLE 1 | Summary of published articles on the neurological wake-up test (NWT) in traumatic brain injury (TBI) and their key findings.

Reference	TBI patients (*n*)	Sedative(s)	Key outcome measure	Main conclusion
(19)	12 (+9 SAH)	Propofol	• ICP increased with 69% and CPP by 5% during the NWT. • MABP and pulse rate increased • Peripheral oxygen saturation unchanged.	NWT increased ICP and MABP
(39)	38 TBI (21 TBI and NWT, 17 TBI controls)	Mainly propofol and remifantanil	• Length of stay and days on mechanical ventilation not significantly altered	No ICU benefit of the NWT
(40)	17	Propofol	• ICP and CPP increased • Interstitial levels of glucose, lactate, pyruvate, glutamate, glycerol, and the lactate/pyruvate ratio unchanged measured by microdialysis. • $SjvO_2$ and $PbtiO_2$ unchanged	No evidence of an exacerbated brain injury by the NWT
(41)	24	Propofol	• ICP and CPP increased • Epinephrine, norepinephrine, and ACTH levels in blood increased • Cortisol in saliva increased • Modest absolute increases of stress hormone levels	NWT induced a biochemical stress response
(42)	TBI $n = 4$ SAH $n = 14$; ICH $n = 2$	Combination of DEX, midazolam, propofol and fentanyl	• 54 NWTs were attempted, 1/3 stopped due to increased ICP. • $PbtiO_2$ decreased in NWT failures. • In only one NWT was neuroworsening detected. • ICP and MABP increased	Many NWTs stopped for safety concerns, no benefit of the test
(43)	242; NWT performed in 96 patients	Propofol	• Early, <24 h, NWT stopped in 40% of patients ($n = 27$) • Reasons for NWT failure was "neurological" in 71% (increased ICP or status epilepticus in 33% of these) or respiratory in 26%	NWT failure associated with subdural hematoma thickness or GCS score <5

ICP, intracranial pressure; CPP, cerebral perfusion pressure; $SjvO_2$, jugular venous saturation; $PbtiO_2$, brain tissue oxygenation; MABP, mean arterial blood pressure; DEX, dexmedetomidine; ICU, intensive care unit; ACTH, adrenocorticotrophic hormone; ICH, intracerebral hemorrhage; SAH, subarachnoid hemorrhage; GCS, Glasgow Coma Scale.

may facilitate use of the NWT due to its shorter half-life and one factor explaining the variable use of the NWT in NCC may be the choice of sedatives in certain centers (44).

Advocates for using the NWT in TBI argue that this test is the only monitoring tool that can reliably detect clinically important neurological improvement or deterioration, including the emergence/exacerbation of focal neurological deficits (45). Clinical changes detected by the NWT could include signs of progressive brainstem involvement or provide clinical evidence for successful surgery of intracranial mass lesions. In addition, based on information obtained by the NWT clinical management may be more aggressive in deteriorating patients, or lead to, e.g., extubation in those showing signs of recovery (16).

The information obtained by the NWT may also facilitate clinical decisions on, e.g., changing ventilator strategies, surgical treatment, or the ordering of neuroradiological investigations. Thus, the indications for the NWT are obvious. There are however numerous other neuromonitoring possibilities in modern NCC that in addition to ICP and CPP monitoring include brain neurochemistry [intracerebral microdialysis (MD)], brain tissue oxygen monitoring ($PbtiO_2$), and jugular venous oxygen saturation ($SjvO_2$) monitoring, among others. These additional tools help to control and maintain intracranial dynamics with the aim to prevent, detect, and treat secondary insults known to exacerbate the primary injury (46, 47). One caveat of neuromonitoring is that although ICP elevations and brain herniation are commonly linked, they can occur independently (48). This means that in, e.g., temporal contusions or following decompressive craniectomy, worsening of the intracranial situation detectable by the NWT may occur without distinctly increased ICP. Since continuous sedation will mask clinical exacerbation, the NWT remains a golden standard for the detection of neurological deterioration even in the presence of advanced neuromonitoring (49).

THE NWT-TECHNICAL ASPECTS AND CONTRAINDICATIONS

For the NWT to be considered, it is imperative that the patient shows stable ICP and CPP values as well as $PbtiO_2$ values at baseline, during continuous sedation. Conversely, the NWT should not be used in patients with ICP and/or CPP problems, or in patients with marked hyperthermia, status epilepticus, and/or barbiturate treatment. A prerequisite for the NWT is obviously that the TBI patient is without sedation at the time of the neurological assessment. Thus, when an NWT is planned, the continuous infusion of sedatives is interrupted although a low dose of analgesics during the wake-up test may preferably be maintained (50). To perform the NWT (**Figure 1**), the patient should be placed in the supine position. The time from interruption of sedation to the NWT may be highly variable (19), and careful monitoring of ICP and CPP during this time is needed. Prior to performing the NWT, the patient should be evaluated to ensure that he/she is sufficiently awakened from the sedation to enable further assessment. Then, always with a watchful eye on the ICP/CPP readings, the patient is requested to obey simple commands (squeeze a hand, move a foot, etc.) and the evaluator scores the response according to the GCS-M. If the patient does not obey commands even after repeated testing, a painful stimulus at the angle of the jaw is delivered and the best GCS-M response (e.g., withdrawal, stereotypic flexion/extension, localization; **Figure 1**) is noted. Neuroworsening, e.g., deterioration of the level of consciousness defined as a drop in the GCS-M score of ≥two points (51), mandates further investigations. In addition, each extremity should be assessed for the presence of focal neurological deficits and the pupil diameter, presence of anisocoria and the direct and indirect pupillary light reflexes be evaluated (**Figure 1**).

STUDIES OF THE NWT IN SEVERE TBI

To date, there are only scarce reports evaluating the NWT in NCC (**Table 1**) and there are no clinical guidelines for using, or avoiding, the NWT. In the only randomized control trial addressing a daily interruption of continuous sedation (DIS) protocol in a small subgroup of TBI patients, significantly decreased days on mechanical ventilation or length of stay in the NCC were not observed (39). However, these results were obtained from only 21 TBI patients who were compared to 17 TBI controls in whom continuous sedation was used. Yet, the duration of mechanical

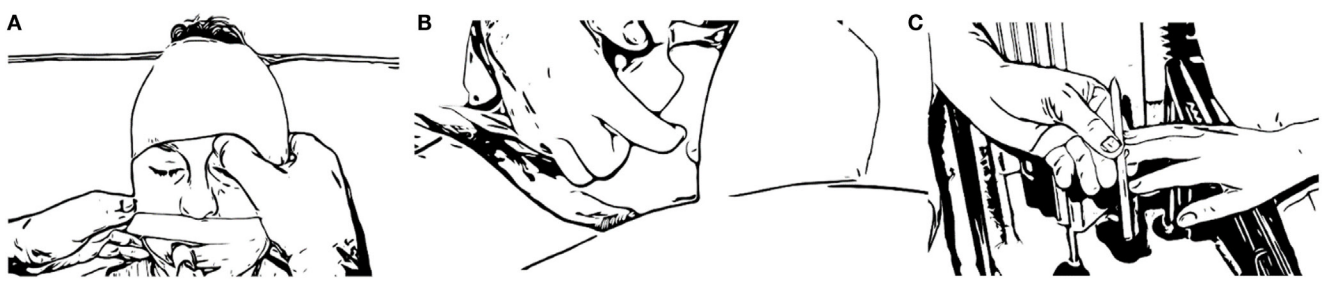

FIGURE 1 | Illustration on how to evaluate the pain response, an integral component of the NWT of unconscious patients unable to obey commands. Apply a steady pressure at the medial aspect of the periorbital rim, at the supraorbital notch **(A)**, or preferably at the angle of the jaw **(B)**. After the pain response has been noted, a peripheral pain stimulus is provided by compressing the fingertips with a pencil **(C)** and, e.g., a localization, withdrawal, flexion, or extension response can be recorded (52). Based on the response of the patient, the motor component of the Glasgow Coma Scale can then be evaluated. The pupillary response to light and the presence of anisocoria as well as any focal neurological deficits are also noted.

ventilation was 7.7 days in TBI patients receiving the DIS protocol vs. 11.6 days in the controls, and the length of NCC stay was 14 vs. 17 days, respectively.

The basic idea of an NWT is to evaluate and detect alterations in the neurological status of TBI patients. The potential benefits or risks associated with the procedure have only been studied in a few reports, most of which are from our own group. In an initial report, 127 NWTs in 12 TBI and 9 subarachnoid hemorrhage (SAH) patients were evaluated (19). In all NWTs, a stress response was observed including transient increases in pulse rate and increased mean arterial blood pressure (MABP). The duration from the interruption of sedation until the NWT could be performed was variable, with a mean of 23 min and a maximal duration of 109 min. The ICP increased by a mean of 69%, from 13 to 23 mmHg, in TBI patients while the CPP showed a non-significant 5% increase during NWT (19). In 9 of the TBI patients, the ICP levels reached >30 mmHg where the highest recorded ICP level was 71 mmHg during the NWT. In two TBI patients, the CPP increased to >130 mmHg. In addition, the CPP levels decreased to <50 mmHg in four TBI patients with the lowest recorded CPP being 29 mmHg. These ICP increases and/or CPP changes were predominately brief and transient, and it was concluded that the NWT procedure was safe in a majority of TBI patients. As mentioned, the ICP and/or CPP changes were marked in a subset of patients where an additional insult to the injured brain cannot be excluded. In addition, even short-lived ICP increases may be associated with a worse outcome in TBI (19, 53).

To address the concern that a secondary insult to the injured brain could be induced by the NWT, 17 patients with severe TBI (11 focal TBI, 6 diffuse/mixed TBI) were studied (40). The effects of the NWT on $PbtiO_2$, $SjvO_2$, and arterial-venous differences (AVDs) of O_2, glucose, and lactate, and interstitial neurochemistry as measured by cerebral MD were evaluated. The $PbtiO_2$ was analyzed in 51 NWTs of 8 TBI patients and remained unaltered and stable throughout the NWT procedure. At baseline, two patients had a $PbtiO_2 < 10$ mm Hg, one of which showed increasing $PbtiO_2$ levels during the NWT. Similarly, the $SjvO_2$ and AVDs were analyzed in six TBI patients for a total of 28 NWTs. No jugular venous catheter readings were exacerbated by the NWT. One patient had a jugular venous saturation <50% on two occasions at baseline, which increased to >60% during both NWTs. Finally, MD was used in 12 TBI patients using the regular perfusion flow rate of 0.3 μL/min in 21 NWTs or, in order to better appreciate any rapid changes potentially induced by the NWT, an increased flow rate of 1.0 μL/min in 28 NWTs. Regardless of the perfusion flow rate, the NWT did not alter interstitial glucose, lactate, glycerol, glutamate, or the lactate/pyruvate ratio. In this and previous reports, the ICP and CPP levels, MABP, and pulse rate were significantly increased by the NWT (19, 40, 41). However, the results of MD, $SjvO_2$, and $PbtiO_2$ monitoring suggested that despite an NWT-induced stress response, no evidence of an additional brain injury was observed (40).

Severe TBI is *per se* accompanied by a systemic biochemical stress response including the release of stress-related hormones such as cortisol and the catecholamines norepinephrine and epinephrine (54–57). Arguably, continuous sedation attenuates this stress response, which aids in controlling ICP. As previously mentioned, one potential risk of using the NWT is the exacerbation of the TBI-induced stress response. NWT-induced changes in plasma adrenocorticotrophic hormone (ACTH), as well as serum norepinephrine and epinephrine levels were evaluated and compared to baseline samples drawn during continuous sedation and prior to NWT. In addition, saliva cortisol was collected by a sublingual swab (41). In 8 patients and 12 NWTs, the catecholamines epinephrine and norepinephrine levels increased by 87.5 and 40.4% from baseline, respectively. For ACTH and cortisol, the NWT-induced increases were 72.5 and 30.7%, respectively. There was no association between the increased levels of these stress hormones and peak ICP or the level of consciousness. Although the NWT significantly increased all stress hormone levels when compared to baseline, their increases in absolute numbers were minor. This study provides an additional argument that the NWT causes a stress response, which however is mild in the majority of TBI patients.

Finally, in a mixed cohort of brain-injured patients of which only four had a severe TBI, interruption of sedation was avoided in 47% of eligible patients due to critical ICP levels, hemodynamic instability, and a need for sedation (42). The authors then performed 54 NWTs, in their article named interruption of sedation trials. Of these, a third of trials could not be completed due to ICP crisis, agitation, desaturation, or a combination of these factors. In addition, reduced $PbtO_2$ levels were commonly observed. In only one completed trial was a neuroworsening detected and it was argued that monitoring with MD enabled the detection of this deterioration prior to the NWT. Although there were only few TBI patients in this study, the results emphasize that not every TBI patient should be subjected to an NWT and that careful risk stratifications and individualized assessments are needed (20, 42, 58). The results of this study were also supported by a retrospective report on the use of the NWT in severe TBI in which failure to complete the test was common (43).

PROS AND CONS OF THE NWT AS A MONITORING TOOL FOR SEVERE TBI

Since one key aim of NCC for TBI is to avoid secondary insults, does the obtained information by the NWT justify the risk of increased ICP and/or decreased CPP? How detrimental is the stress response, observed in virtually all NWT studies, and are there any identifiable clinical benefits of the test (**Table 2**)? Interruption of continuous sedation has not been shown to cause long-term psychiatric problems such as posttraumatic stress disorder or recall of event (31). In experienced hands, the NWT-induced stress response is mild in the majority of patients (19), at least in those TBI patients who are stable at baseline, and adverse effects such as self-extubation are rare. Thus, when the ICP, CPP, and/or $pBTiO_2$ recordings assessed prior to sedation interruption are within accepted limits, the NWT could be considered.

TABLE 2 | Summary of some important pros and cons on the neurological wake-up test in traumatic brain injury.

Pro	Con
Detection of changes in neurological status leads to more active management	Induces a stress response with increased ICP, changes in CPP, hypertension
Reduced risk for ventilator-associated pneumonias, reduced ICU stay and less time on ventilator?	No clinical benefit over multimodality monitoring
An important clinical decision tool	Increases brain metabolism and oxygen consumption

ICU, intensive care unit; ICP, intracranial pressure; CPP, cerebral perfusion pressure.

There is a complete lack of Level I evidence for using, or refraining from using, the NWT in NCC and its use may predominately be based on personal preferences and/or experience as well as locally adopted guidelines and traditions. In the previous study by Helbok and colleagues, evidence of a new focal neurologic deficit was found only in one SAH patient and in no TBI patient, although in this study only four TBI patients were included (42). Surprisingly, systematic analysis of the information achieved by the NWT in TBI and what clinical decisions are made based on this information is rare. Such studies should be feasible to design and be crucial in interpreting the role for NWTs in TBI management. If the NWT does not provide information needed for important clinical decisions, its use cannot be justified. Conversely, if the NWT leads to more active management, detection of relevant causes for neuroworsening and/or improvement, and guides clinical decisions then the NWT-induced stress response can be motivated if the patient is carefully monitored during the procedure.

There are also many arguments against the NWT. As stated in the article by Helbok and colleagues, the NWT may only rarely add clinical information of importance over other monitoring tools (42). In addition, the NWT-induced stress response is likely to increase cerebral metabolism and oxygen consumption, factors not desirable in the vulnerable TBI patient (20). A valid argument against the routine implementation of the test is also the exclusion of patients unstable at baseline, since these individuals may be those in whom the NWT would add the most useful information. Although sedation *per se* has never been shown to positively influence outcome, it is clearly a treatment in itself for ICP and CPP control, stress reduction, and attenuation of cerebral energy metabolism (24, 50, 59).

Due to the lack of solid data, the central question remains—how often can the NWT detect an altered neurological condition that will influence patient management? This question calls for additional studies. If no clinical benefits can be identified from the NWT, there are other available sedation algorithms in combination with sedation scales that may reduce the risk of over-sedation (42). Recently, it was suggested that in all patients at risk for ICP elevations, in those undergoing active temperature lowering therapies and in those treated for refractory status epilepticus the NWT should be avoided. If these factors are not present, NWTs/interruption of sedation protocols can be used as in general ICU care (23). It appears feasible that modern multimodal monitoring and NWT may co-exist, and that other monitoring tools can be used to define in what TBI patients it is safe, or unsafe, to perform an NWT.

CONCLUSION

The aim of this review was to assess the available literature on the use of the NWT as a monitoring tool in the NCC management of TBI patients. To date, there are no strong arguments for a clinical benefit of the NWT in severe TBI patients. An obvious goal of continuous sedation is also the reduction of cerebral energy metabolic demands in severe TBI. In the majority of evaluated studies, the NWT is associated with a variable systemic stress response. However, there are no data clearly showing that this stress response results in a significant secondary brain injury. Patients with unstable ICP and/or CPP levels, hyperthermia and/or status epilepticus should not be subjected to the test. In others, when used by personnel experienced in the interruption of sedation required for the NWT, the test may in medically stable TBI patients provide useful clinical information such as neuroworsening or neuro-improvement and be used in daily clinical decision-making. There is thus an argument for implementing the NWT in management protocols for selected TBI patients. In summary,

- From a scientific perspective, there is neither evidence against the use of the NWT nor in its favor.
- The NWT is associated with a stress response, the consequences of which have not been fully elucidated. To date, there is no clear evidence for a secondary brain injury induced by the NWT.
- Factors such as local management traditions, experience of the nursing staff and/or the choice of sedatives appears to decide the use and frequency of the NWT.
- An individualized assessment based on neuromonitoring and neuroimaging parameters is needed to decide in which patient the NWT is safe.
- The choice is not between multimodality monitoring and the NWT; TBI management strategies may well include a combination of both.
- A study systematically evaluating the clinical decision-making based on information obtained by an NWT appears feasible and could enhance the knowledge of the pros and cons as well as define the role of the NWT in modern-day NCC.

AUTHOR CONTRIBUTIONS

The author confirms being the sole contributor of this work and approved it for publication.

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REVIEW
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Cerebral Microdialysis Monitoring to Improve Individualized Neurointensive Care Therapy: An Update of Recent Clinical Data

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Cerebral microdialysis (CMD) allows bedside semicontinuous monitoring of patient brain extracellular fluid. Clinical indications of CMD monitoring are focused on the management of secondary cerebral and systemic insults in acute brain injury (ABI) patients [mainly, traumatic brain injury (TBI), subarachnoid hemorrhage, and intracerebral hemorrhage (ICH)], specifically to tailor several routine interventions—such as optimization of cerebral perfusion pressure, blood transfusion, glycemic control and oxygen therapy—in the individual patient. Using CMD as clinical research tool has greatly contributed to identify and better understand important post-injury mechanisms—such as energy dysfunction, posttraumatic glycolysis, post-aneurysmal early brain injury, cortical spreading depressions, and subclinical seizures. Main CMD metabolites (namely, lactate/pyruvate ratio, and glucose) can be used to monitor the brain response to specific interventions, to assess the extent of injury, and to inform about prognosis. Recent consensus statements have provided guidelines and recommendations for CMD monitoring in neurocritical care. Here, we summarize recent clinical investigation conducted in ABI patients, specifically focusing on the role of CMD to guide individualized intensive care therapy and to improve our understanding of the complex disease mechanisms occurring in the immediate phase following ABI. Promising brain biomarkers will also be described.

Keywords: microdialysis, traumatic brain injury, subarachnoid hemorrhage, cerebral metabolism, ischemia, hypoxia, biomarkers, neurointensive care

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INTRODUCTION

Cerebral microdialysis (CMD) has progressively evolved from a tool for clinical research into an additional brain monitoring modality to guide neurointensive care (1, 2). Evidence has accrued over the last years that CMD monitoring—in combination with other modalities such as intracranial pressure (ICP) and brain tissue PO_2 ($PbtO_2$), so called multimodal monitoring—may help guiding individualized intensive care therapy of comatose brain-injured patients, mainly after traumatic brain injury (TBI) and aneurysmal subarachnoid hemorrhage (SAH) (3, 4). Clinical utility of CMD has been particularly shown for the management of "secondary" cerebral insults, i.e., the number of pathological events that occur in the early phase following acute brain injury (ABI). The use of CMD has contributed to better define therapeutic thresholds for several routine interventions, such as cerebral perfusion pressure (CPP) optimization, oxygen therapy, red blood cell

transfusion (RBCT), and metabolic control (blood glucose and nutrition). Exploration of the injured brain with CMD has also greatly contributed to better understand important post-injury mechanisms—such as energy dysfunction, hyperglycolysis, cortical spreading depressions, subclinical seizures, or brain edema—and to identify potential novel biomarkers of injury and prognosis. Recent reviews focused on specific technical aspects related to CMD monitoring, both in terms of the catheters and microdialyzate analyser technology (1). The scope of this review was to summarize recent clinical investigation conducted in neurocritical care patients, aiming to discuss the role of CMD to guide individualized intensive care therapy and to improve our understanding of the complex disease mechanisms occurring in the immediate phase following severe brain injury. We also describe emerging data on the potential utility of CMD to assess novel biomarkers of injury, as well as its role in interventional and pharmacological studies. We mainly focused our review on clinical studies published during the last 5 years (January 2012 to September 2017) and performed in patients with ABI, including TBI, SAH, and ICH.

INTERPRETATION OF CMD VARIABLES AND REFERENCE VALUES

In clinical practice, CMD biomarkers (generally sampled every hour and immediately analyzed at the bedside) should always be interpreted in the context of monitor location, type of injury, and patient clinical condition. Based on accrued clinical data over the last decade linking glucose and lactate/pyruvate (L/P) ratio with principal outcomes after ABI, compared to glutamate and glycerol, the 2015 CMD Consensus proposed to interpret CMD biomarkers in a tiered fashion and to use primarily CMD L/P ratio and glucose as step 1 to guide clinical interventions (2). Abnormalities of CMD L/P ratio and glucose reflect the complex pathophysiology underneath ABI; therefore, correct interpretation require integration of other monitored variables such as ICP and $PbtO_2$.

Elevated CMD lactate and L/P ratio may be a marker of inadequate cerebral blood flow (CBF) and/or oxygen delivery. In this context, dramatic increases may be observed, which are associated with a concomitant decrease in CMD pyruvate and glucose. Given that cerebral circulation and/or oxygenation are impaired, ICP/CPP and/or $PbtO_2$ values will be abnormal.

However, CMD lactate and L/P ratio may be elevated because of other mechanisms than ischemia or hypoxia (5). Cerebral energy dysfunction/failure has been described despite CBF and brain tissue oxygenation being normal (6, 7), whereby elevations of CMD lactate and L/P ratio may be predominantly attributable to increased glycolysis or mitochondrial dysfunction (impairment of oxygen utilization or cytopathic hypoxia) (8, 9). In this context, pyruvate may be normal or elevated, and elevations of CMD lactate and L/P ratio are of a lesser extent than during frank ischemia/hypoxia.

Low CMD glucose, therefore, may be related to cerebral energy dysfunction (10). On the other hand, apart from cerebral causes (ischemia/hypoxia or energy dysfunction), inadequate systemic glucose, because of intensive insulin therapy to maintain strict glycemic control, may cause further reductions of CMD glucose (11, 12).

To direct individualized intensive care therapy, it is therefore important to consider CMD L/P ratio rather than lactate alone, to look for dynamic changes and trends of both CMD L/P ratio and glucose, and finally to take into account additional monitor modalities (ICP/$PbtO_2$), according to the modern paradigm of multimodality monitoring (13, 14).

Interpretation of absolute values is also dependent on probe location in an area of normal-appearing vs. around a lesion (e.g., hematoma or contusion) (2, 15). Also, a recent study in SAH patients suggests that delayed cerebral ischemia may be detected only when the probe is located within a brain area later affected by secondary infarction, which may justify the use of implantation guidelines (16).

In **Figure 1**, we propose an algorithm for interpretation of CMD abnormalities, centered on low CMD glucose as starting point of the clinical reasoning.

As for reference values, L/P ratio >25 is considered abnormal (impaired cerebral oxidative metabolism), while L/P ratio >40 is the critical level above which brain energy crisis is defined. The reference level for CMD glucose is still debated, but probably lies at 1 (±0.15) mmol/L (17).

CMD TO GUIDE INDIVIDUALIZED INTENSIVE CARE THERAPY

Optimization of Substrate Supply

The CMD technique allows semicontinuous monitoring of cerebral glucose metabolism and of the interactions between blood and brain glucose in humans under conditions of varying glycemia (18). Glucose is the main substrate for the brain. However, in the aftermath of injury, the brain's ability to use glucose may be reduced (19). Cerebral extracellular glucose may be limited (10, 20), therefore, enabling adequate glucose supply in ABI patients appears crucial to attenuate further brain damage (21). Following the two large single-center studies by van Den Berghe and colleagues in the early 2000 (22, 23), suggesting that tight glycemic control may benefit general critically ill patients, Vespa and colleagues were the first to show that actually this so-called intensive insulin strategy was associated with an increased prevalence of low CMD glucose and elevated LPR (24). This CMD study was concomitant to another outcome study by the Leuven's group showing that, at the contrary, strict glycemic control may also benefit the outcome of neurointensive care patients (25). Additional CMD studies from several groups subsequently confirmed the seminal clinical investigation by Vespa and colleagues, showing that indeed strict glycemic control might reduce cerebral glucose availability and aggravate cerebral energy dysfunction (11, 26–31). Given the results of the multicentre NICE-SUGAR study, which did not confirm substantial outcome benefit for intensive vs. moderate blood glucose control both in the general ICU population (32, 33), and in the *post hoc* analysis of neurotrauma patients (34), a strategy of liberal glycemic control (7–10 mmol/L) was generally felt as safer in critically neurological patients by international recommendations (35). Indeed, using a cross-over design that alternated tight to moderate glycemic control, Vespa confirmed

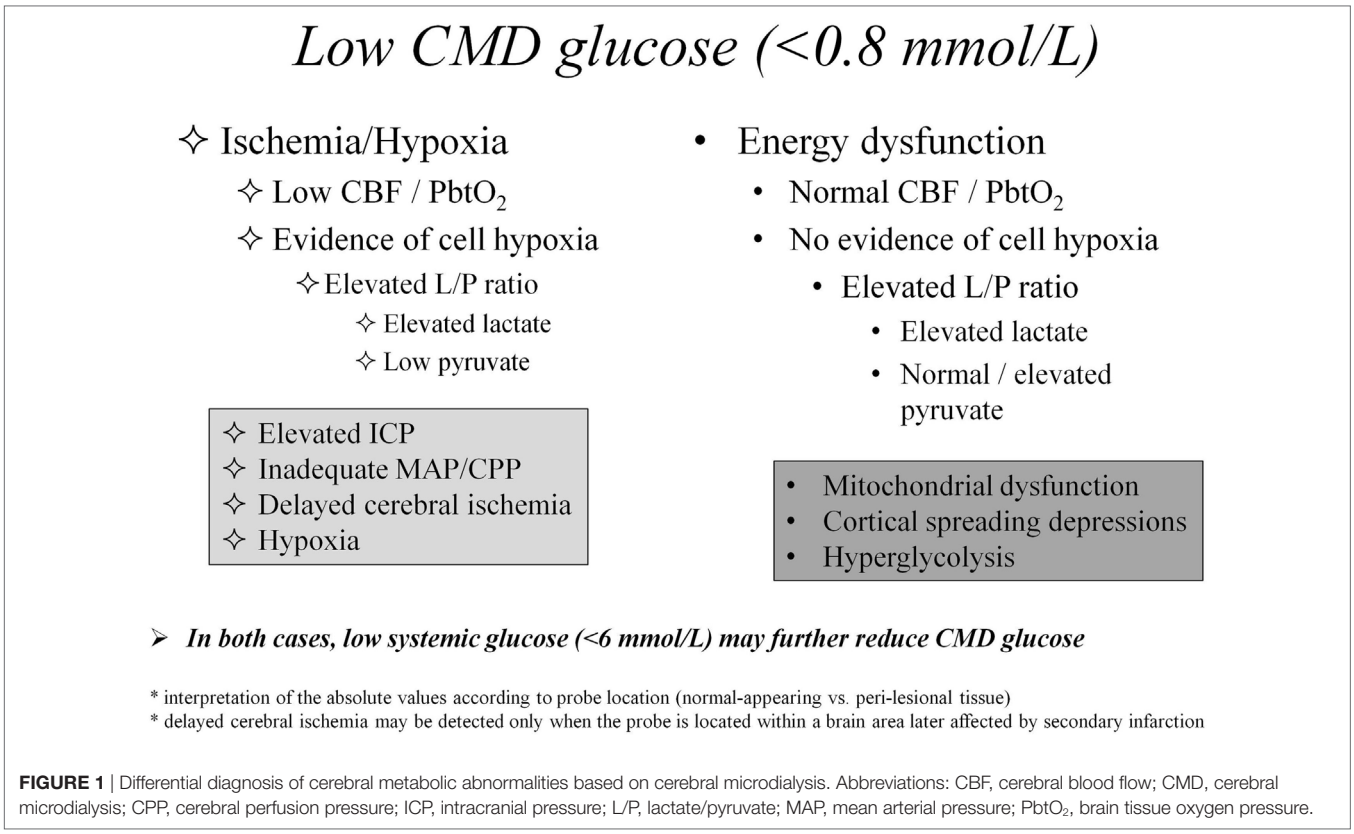

FIGURE 1 | Differential diagnosis of cerebral metabolic abnormalities based on cerebral microdialysis. Abbreviations: CBF, cerebral blood flow; CMD, cerebral microdialysis; CPP, cerebral perfusion pressure; ICP, intracranial pressure; L/P, lactate/pyruvate; MAP, mean arterial pressure; $PbtO_2$, brain tissue oxygen pressure.

previous findings that intensive insulin therapy was associated with increased metabolic distress, as judged by lower CMD glucose and higher CMD L/P ratio during tight glycemia (12).

The glycemic control controversy illustrates how CMD monitoring has contributed to the actual progresses of intensive care therapies, and how physiologically oriented studies may influence our practice, especially in the field of neurointensive care where "true" evidence-based medicine derived from RCT is often lacking. A recent example of such approach was provided by the Innsbruck group led by Helbok: the authors found that rapid effective institution of enteral nutrition was associated with an increase in CMD glucose that was directly dependent on the magnitude of increase of blood glucose (36), reinforcing the recommendations for the early institution of enteral feeding in neurointensive care patients.

The Consensus on CMD suggests the use of CMD monitoring for the detection and treatment of low cerebral glucose, and to guide systemic glucose management and insulin use (2).

Optimization of Cerebral Perfusion

CMD markers—such as glucose and L/P ratio—may be good surrogate markers of CBF, and indeed this has recently been confirmed by several clinical studies combining microdiaylsis with brain imaging, both in patients with SAH (37–39) and TBI (40). A recently published small observational cohort study illustrated the potential value of CMD monitoring to help detecting cerebral hypoperfusion in comatose aSAH patients, in whom, the clinical examination was unreliable (37). This study stressed the importance of following dynamic trends over time of both CMD L/P ratio and glucose for the timely detection of secondary cerebral ischemic insults. It also confirmed the potential value of CMD biomarkers to avoid low CPP by adjusting CPP thresholds individually in comatose ABI patients (16, 41–43). Indeed, Bouzat and colleagues found that the addition of CMD (in combination with $PbtO_2$) to ICP monitoring significantly improved the accuracy of detecting secondary hypoperfusion in patients with severe TBI (40).

The use of CMD monitoring to optimize CCP in order to prevent/avoid ischemia is recognized as potentially clinically useful for TBI and SAH patients by the Consensus on CMD (2).

Optimization of Oxygen Transport: Blood Transfusion and Oxygen Therapy

Red Blood Cell Transfusion

Whether restrictive or more liberal thresholds for hemoglobin and RBCT should be used in neurointensive care is still debated, given the lack of randomized clinical trials in this setting. It is possible that the therapeutic approach may vary individually, according to the extent of injury; therefore, patients with more severe brain insults may benefit from higher hemoglobin (Hgb) levels (44, 45). Indeed, low Hgb <9 g/dL was shown to be associated with increased CMD markers of cerebral ischemia (elevated L/P ratio and low CMD glucose) (46, 47). The question is whether enhancing cerebral oxygen transport with RBCT may reduce cerebral damage: RBCT might improve $PbtO_2$ in the majority (although not all) of patients (48, 49); however, improved $PbtO_2$

did not translate into a clinically relevant benefit on cellular metabolism, as quantified by the non-significant amelioration of CMD L/P ratio (50, 51).

Oxygen Therapy

In various subsets of critically ill patients, including those with ABI, increasing inspired fraction of oxygen (FiO_2) to achieve arterial hyperoxia (arterial partial pressure of oxygen, PaO_2, >150 mmHg) was associated with worse outcome (52). Whether or not hyperoxia is beneficial after ABI remains controversial. Physiological studies testing the effect of hyperoxia on CMD biomarkers were conducted predominantly on TBI patients. Improving $PbtO_2$ by way of normobaric hyperoxia may reduce L/P ratio (53, 54), although this effect seems of limited clinical relevance (55). When using CMD glutamate as a marker of increased excitotoxicity, Quintard and colleagues found an association between normobaric hyperoxia and increased cerebral glutamate (56). Recently, two prospective single-center trials brought additional important insights. Ghosh and colleagues, testing 120-min normobaric hyperoxia challenge in the acute phase (24–72 h) of TBI (16 patients; using an advanced multimodal monitoring, including $PbtO_2$, CMD, near-infrared spectroscopy, and transcranial Doppler) found that hyperoxia was associated with an improvement of L/P ratio, as well as all other oxygenation and perfusion parameters, consistent with increased aerobic cerebral metabolism and better cellular redox state (57). Vidal-Jorge and colleagues in an elegant study using CMD to sample biomarkers of oxidative stress (8-iso-Prostaglandin F2α) found that increasing FiO_2 to 1.0 for 4 h resulted in marked reduction in both CMD lactate and CMD L/P ratio only in patients with more severe injury, as defined by a CMD lactate >3.5 mmol/L, but did not change energy metabolism in the whole group of patients (58). Furthermore, hyperoxia caused a significant increase in 8-iso-PGF2α in patients in whom oxidative stress was detected at baseline, but not in those without (58).

Rockswold and colleagues, using a Phase II observational design, found that hyperbaric oxygen therapy [1 h at 1.5 atmospheres absolute (ATA)], followed by 3-h normobaric hyperoxia (100% FiO_2 at 1.0 ATA) was effective in improving CMD L/P ratio and glycerol after TBI, both in relatively uninjured brain as well as in peri-contusional tissue; tissue benefit translated into better outcome in this study (59).

Overall, CMD has evolved over time as a tool that may help guiding individualized targeted therapy at the bedside in ABI patients and to test the physiologic response to a specific intervention (**Table 1**).

CMD to Test the Efficacy of Pharmacological Interventions

Although it was not validated so far in large multicentre studies, CMD biomarkers such as CMD L/P ratio and glucose are associated with patient prognosis, at least in TBI patients (60). Therefore, it is conceivable to use CMD metabolites as surrogate outcome endpoints to test therapeutic efficacy in Phase II clinical trials.

Examples of therapies tested in studies using CMD biomarkers as surrogate outcome endpoints include:

- nitric oxide synthase inhibition (61)
- recombinant human interleukin-1 receptor antagonist (62)
- antiepileptic drugs (63, 64)
- focally perfused succinate (65)
- intravenous hypertonic lactate (66, 67)
- sedation (68).

Measuring the concentrations of drug molecules in the brain extracellular fluid appears superior to cerebrospinal fluid or plasma to test the ability to effectively deliver pharmacological agents across the blood–brain barrier into the brain and is an important step in the development of central nervous system therapies. CMD sampling can give valuable pharmacokinetic information of variations with time in drug concentrations of brain interstitial tissue versus plasma and may help in designing future therapies (69, 70), or to test drug penetration of several pharmacologic agents, such antimicrobials (71, 72) or antiepileptic drugs (63, 64).

CMD TO EXPLORE THE COMPLEX ABI PATHOPHYSIOLOGY

Alterations of cerebral perfusion/oxygenation (73–75) and brain energy metabolism (9, 19, 20, 76–82) are important determinants of ABI. However, additional mechanisms are implicated

TABLE 1 | Examples of ICU interventions guided by CMD.

	Energy supply		Cerebral Perfusion	Oxygen transport	
				FiO_2, PaO_2	**(Hgb)**
Therapeutic intervention	Insulin therapy	Enteral nutrition	Intracranial pressure/CPP targets	NBHO	RBCT
Risks	↓ CMD glucose <0.7 mmol/L	↑ blood glucose	Ischemia, ↓ CPP	Increased excitotoxicity	Ischemia/hypoxia vs. RBCT-related complications
Benefits	Optimal glycemia	↑ CMD glucose	Optimal CPP	Optimal PaO_2	Optimal (Hgb)
CMD targets	CMD glucose >0.7 mmol/L		↓ L/P ratio ↑ CMD glucose	↓ L/P ratio	↓ L/P ratio

CMD, cerebral microdialysis; CPP, cerebral perfusion pressure; Hgb, hemoglobin; FiO_2, fraction of inspired oxygen; ICU, intensive care unit; L/P lactate/pyruvate; NBHO, normobaric hyperoxia; PaO_2, arterial partial pressure of oxygen; RBCT, red blood cell transfusion.

in post-injury pathophysiology and CMD has contributed to elucidate some of these mechanisms (**Figure 2**). In this context, CMD catheters with larger membrane cut-off (100 kDa) than the standard ones (20 kDa) may have great utility for the identification and bedside follow-up of biomarkers of injury (e.g., cytokines, metallo-proteases) and recovery (e.g., markers of neurodegeneration) in specific pathologies (70, 83).

The Link between Energy Dysfunction and Electrographic Crisis

Non-convulsive seizures and pseudo-periodic discharges might amplify secondary cerebral damage in the setting of ABI: using an elegant approach combining CMD with surface and intracortical electro-encephalography, Vespa and colleagues recently established a mechanistic link between seizures and metabolic crisis (84). This study is another example of how CMD can be used to monitor complex and concealed mechanisms but also to test the efficacy of future interventions aimed at specifically targeting seizure suppression.

Along the same line, pathological spreading depressions, which are frequently seen in TBI and SAH patients (85), cause significant local cerebral metabolic disturbances (reduced CMD glucose, elevated CMD LPR, and glutamate) (86–88); therefore, it is conceivable to use CMD as target for future interventional trials aimed at specifically treating spreading depressions.

Early Brain Injury and Cerebral Edema

Microdialysis studies have contributed to better characterize the exact nature of cerebral edema in different pathologies and to differentiate between cellular (or cytotoxic) and vasogenic edema. Alterations in the ionic profile of the extracellular space [main electrolytes (Na^+, K^+, Cl^-) and amino-acids like taurine] correlate with cellular edema in patients with diffuse injury after TBI (89–92). Matrix metalloproteases (MMP) are important pathogenic determinants of blood–brain barrier breakdown and vasogenic edema: using 100 kDa catheters, which allows sampling of larger molecules, elevated CMD MMP have been observed in patients with focal parenchymal hemorrhages following TBI and SAH (93–97). These physiology studies contribute to better refine future treatments of brain edema, according to the specific pathology.

Inflammation and Oxidative Stress

Using CMD has allowed the exploration of cytokine and chemokine profile after ABI (98–101), as well as to follow the dynamic changes in brain extracellular fluid of other biomarkers of inflammation (102), oxidative stress (NAA, isoprostane) (103, 104), and endothelial dysfunction (nitric oxide) (105), which may also be potential surrogate endpoints for interventional studies (58). Two recent scoping systematic reviews have addressed the potential value of microdialysis cytokines in severe TBI and poor-grade SAH (106, 107): although preliminary studies support feasibility of measurements and associations of CMD cytokines with tissue and neurophysiologic outcomes, evidence is very limited and further larger studies need to be conducted.

Neurodegeneration

Markers of axonal degeneration—such as tau, β-amyloid, neurofilament light-chain (NfL), and neurofilament heavy chain (NfH)—have been the focus of recent clinical investigation, often in combination with magnetic resonance imaging, to better characterize posttraumatic axonal injury acutely in the intensive care unit (108–113). Preliminary data also established a potential link between tau protein and early brain injury following SAH (114, 115). Providing the reproducibility of these biomarkers is confirmed in larger scale studies, such approach holds great promise for early prognostication (to complement clinical and radiological information) and for a pathology-based patient selection to optimize future pharmacological interventional studies.

Table 2 summarizes main results of clinical CMD studies and their potential implications and clinical utility.

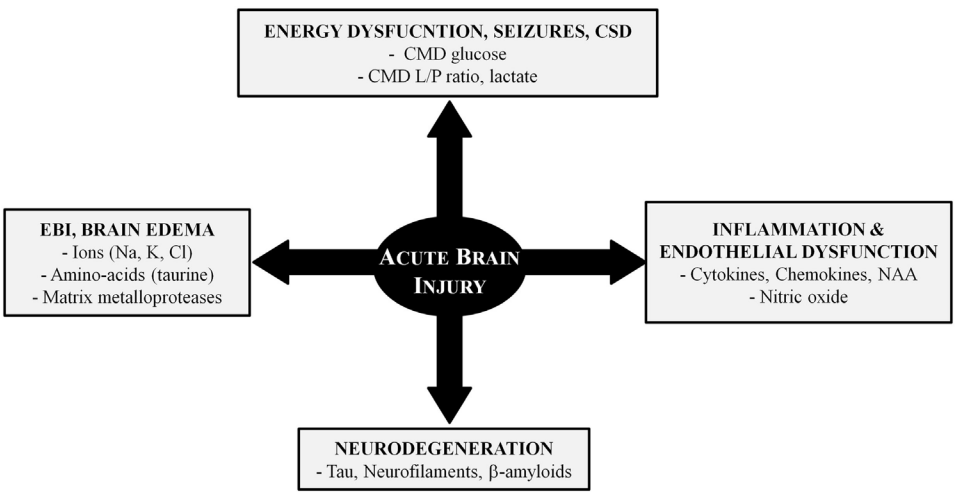

FIGURE 2 | Pathophysiology of acute brain injury: the role of cerebral microdialysis. Abbreviations: CMD, cerebral microdialysis; CSD, cortical spreading depressions; EBI, early brain injury; L/P, lactate/pyruvate; NAA, n-acetyl aspartate.

TABLE 2 | Summary of clinical CMD studies.

Studies	Summary of main results	Clinical utility	Reference
Observational studies			
Glycemic control	Tight (4–6 mM) vs. moderate (6.1–8 mM) glycemic control is associated with more episodes of low $glucose_{CMD}$	Management of insulin	(11, 12, 26–31)
Cerebral perfusion	Cerebral hypoperfusion is associated with increased cerebral metabolic distress (high L/P_{CMD}/low $gluc_{CMD}$)	Early ischemia detection Targeted CPP therapy	(37, 42, 43)
Hemoglobin level	Anemia (Hgb <9 g/dL) is associated with increased cerebral metabolic distress	Management of RBCT	(46, 47, 50, 51)
Oxygen therapy	NBHO (2–4 h) is associated with improved LPR_{CMD} NBHO benefit mostly when baseline $lactate_{CMD}$ >3.5 mM HBOT is associated with improved L/P_{CMD}	Targeted management of PaO_2/FiO_2	(57–59)
Interventional studies			
NOS inhibitors	NOS inhibition (i.v.) does not affect cerebral metabolism	Potential for CMD biomarkers to be used as surrogate efficacy endpoints in phase II clinical trials	(61)
rh IL-1 ra	rh IL-1ra (i.v.) does not affect cerebral metabolism		(62)
Hypertonic lactate	Hypertonic lactate (i.v.) is associated with $glucose_{CMD}$ increase		(66, 67)
Succinate	Succinate (i.c.) is associated with reduced cerebral metabolic distress		(65)
Mechanistic studies			
Seizures	Electrographic seizures are associated with increased cerebral metabolic distress	Monitoring and testing the efficacy of future interventions targeted at reducing seizure and CSD	(84)
CSD	CSD are associated with low $glucose_{CMD}$		(86, 87)
Brain edema	Cellular edema is associated with increased Na^+_{CMD}, K^+_{CMD}, and $taurine_{CMD}$ Vasogenic edema is associated with increased MMP_{CMD}	Targeted therapy of brain edema based on disease pathology	(90–92, 96, 97)
Neuroinflammation	Identification of several cytokines (including IL-1ra, IL-6, IL-8, and TNF-α) involved in the complex inflammatory cascade following acute brain injury	Development of therapeutics targeted at attenuating the inflammatory cascade	(106, 107)
Neurodegeneration	Relationship of tau and NfL with MRI axonal degeneration and patient outcome	Characterization of disease neuropathology Patient selection for interventional studies targeted at reducing neurodegeneration	(108, 109)

CMD, cerebral microdialysis; CPP, cerebral perfusion pressure; CSD, cortical spreading depression; FiO_2, fraction of inspired oxygen; HBOT, hyperbaric oxygen therapy; Hgb, hemoglobin; i.c., intracerebral; IL, interleukin; i.v., intravascular; L/P lactate/pyruvate ratio; MMP, matrix metalloproteases; MRI, magnetic resonance imaging; NBHO, normobaric hyperoxia; NfL, neurofilament light chain; NOS, nitric oxide synthase; PaO_2, arterial partial pressure of oxygen; ra, receptor antagonist; RBCT, red blood cell transfusion; rh, recombinant human; TNF, tumor necrosis factor.

IMPLEMENTATION IN THE INTENSIVE CARE UNIT

Barriers to the widespread implementation of CMD are numerous, including costs, human resources, and the complexity of the technique (especially with respect to 100 kDa catheters) (1). These barriers may explain why CMD monitoring is still not in use in the majority of centers, as judged by a recent National survey on multimodal monitoring conducted in the UK (116). Recent consensus guidelines for the use of CMD in acute brain pathologies (2, 15) and the increased application of CMD in other acute contexts, e.g., anoxic-ischemic (117) or hepatic encephalopathy (118), may contribute to a broader implementation of this technique. The future of CMD is constantly evolving: technical refinements and the potential for automated near real-time continuous measurements may increase the performance and the accuracy of the technique (119–121), thereby facilitating the utilization in the intensive care unit.

CONCLUSION

Cerebral microdialysis is an important neuromonitoring tool that is increasing used at the bedside in combination with ICP and PbtO2 to guide therapy individually in brain-injured patients. Recent consensus on microdialysis monitoring may help optimizing protocols for microdialysis implementation in neurocritical care. Over the last decade, clinical investigation using microdialysis have contributed to better understand pathogenic mechanisms involved in secondary brain damage, such as cerebral edema, energy dysfunction, cortical spreading depression, neuroinflammation, and help refining novel therapeutic approaches, and drug effects on downstream targets. Future improvements of CMD technology may further enhance applicability.

AUTHOR CONTRIBUTIONS

LC drafted the manuscript and **Table 1**. PB drafted the manuscript and the figures. MO drafted and revised the manuscript, and drafted the Figures and **Table 2**.

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REVIEW
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Clinical Use of Cerebral Microdialysis in Patients with Aneurysmal Subarachnoid Hemorrhage—State of the Art

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Objective: To review the published literature on the clinical application of cerebral microdialysis (CMD) in aneurysmal subarachnoid hemorrhage (SAH) patients and to summarize the evidence relating cerebral metabolism to pathophysiology, secondary brain injury, and outcome.

Methods: *Study selection*: Two reviewers identified all manuscripts reporting on the clinical use of CMD in aneurysmal SAH patients from MEDLINE. All identified studies were grouped according to their focus on brain metabolic changes during the early and subacute phase after SAH, their association with mechanisms of secondary brain injury and outcome.

Results: The review demonstrated: (1) limited literature is available in the very early phase before the aneurysm is secured. (2) Brain metabolic changes related to early and delayed secondary injury mechanisms may be used in addition to other neuromonitoring parameters in the critical care management of SAH patients. (3) CMD markers of ischemia may detect delayed cerebral ischemia early (up to 16 h before onset), underlining the importance of trend analysis. (4) Various CMD-derived parameters may be associated with patient outcome at 3–12 months, including CMD-lactate-to-pyruvate-ratio, CMD-glucose, and CMD-glutamate.

Conclusion: The clinical use of CMD is an emerging area in the literature of aneurysmal SAH patients. Larger prospective multi-center studies on interventions based on CMD findings are needed.

Keywords: subarachnoid hemorrhage, neuromonitoring, cerebral microdialysis, brain metabolism, treatment

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INTRODUCTION

First reports on monitoring of brain metabolism using cerebral microdialysis (CMD) in patients with subarachnoid hemorrhage (SAH) date back to the year 1992 (1). Despite the long-standing availability of this technique, recommendations for treatment decisions based on CMD monitoring have just been recently published as a consensus statement by clinical experts (2).

Cerebral microdialysis has improved our understanding of pathophysiological mechanisms of early and delayed brain injury in SAH patients by providing metabolic information derived from brain tissue on a cellular level, in addition to intracranial pressure (ICP), cerebral perfusion pressure (CPP), cerebral blood flow (CBF), brain tissue oxygen tension ($P_{bt}O_2$), and electrographic

monitoring (electroencephalography and electrocorticography). So far, changes in brain metabolism have been associated with known complications after SAH and may also help to detect impending secondary brain injury early or before they have evolved into irreversibility. Moreover, abnormalities in CMD-derived parameters have been linked to poor brain tissue and functional outcome and may therefore be integrated in the multimodal approach of neuroprognostication. In addition, the effect of commonly applied pharmacological and non-pharmacological treatments on brain metabolism can be studied on an individual level, thereby enhancing the concept of personalized medicine in neurocritical care patients.

Microdialysis Methodology

The principle of CMD is to mimic a capillary blood vessel in the brain for the assessment of local cerebral metabolism (3). A tubular semi-permeable membrane on the tip of the CMD catheter is perfused with an isotonic or colloid-enriched fluid that equilibrates with the extracellular space by simple diffusion. All molecules small enough to pass the membrane follow their electro-chemical gradient into the tube. Established catheters have a membrane length of 1 cm and pore sizes of either 20 or 100 kDa, which do not show differences in the recovery of small molecules (4). A perfusion speed of 0.3 µl/min is recommended in clinical use, which leads to a relative recovery rate (dialyzate concentration divided by true concentration) of about 70% for the most commonly assessed molecules (5).

Criteria for CMD monitoring are not well defined. It can be used as part of the "multimodal neuromonitoring bundle" (**Figure 1A**) in ventilated ("poor-grade") patients or in patients with a secondary neurological deterioration. As a primary monitoring device, the probe should be placed in the frontal lobe (anterior/middle cerebral artery watershed), ipsilateral to the ruptured aneurysm, or the maximal blood clot load. When used in patients with secondary deterioration, location can also be guided by local practice to identify tissue at risk (for example, by CT perfusion or transcranial ultrasound) (2, 6). The catheter can be inserted into the brain tissue either by using a cranial access device (bolt) or by tunneling (**Figure 1A**), penetrating the skull *via* a craniotomy (**Figure 1B**) or a twist drill hole. The dialyzate of the first hours should be discarded due to the insertion trauma and dilution effect of the flush sequence filling the system.

Interpretation of Data

Concentrations of CMD parameters represent the local metabolic environment surrounding the membrane and cannot be extrapolated to other regions of the brain. Knowledge of catheter location is therefore mandatory for data interpretation. The gold tip of the CMD probe is visible on head CT (**Figure 2B**), thus its exact location in the brain can be determined and classified by the monitored tissue (gray vs. white matter), lobe, or by the spatial relation to focal pathologies (intralesional/perilesional vs. radiologically normal-appearing brain tissue). The dependency of molecular concentrations on probe location argues for the interpretation of temporal dynamics (trend analysis) in addition to absolute values. Calculating ratios of different CMD parameters (e.g., CMD-lactate-to-CMD-pyruvate-ratio, CMD-LPR) creates variables independent of absolute concentrations and recovery.

The dialyzate is sampled into microvials and analyzed for point of care parameters including CMD-glucose, CMD-lactate, CMD-pyruvate, CMD-glutamate, and CMD-glycerol at the patient's bedside. A measurement interval of 1 h is commonly used in clinical practice.

Glucose is an important energy substrate for neuronal tissue. Its concentration in the brain depends on systemic supply, diffusivity in the brain tissue, and local consumption. In the process of aerobic glycolysis, it is metabolized to pyruvate and further converted into acetyl-coenzyme A, which is used for mitochondrial energy production. Under conditions of brain tissue hypoxia or mitochondrial dysfunction, pyruvate is fermented into lactate. The LPR reflects the cytoplasmatic redox state and is a marker of anaerobic metabolism and/or mitochondrial dysfunction. The concept of mitochondrial dysfunction arose from observations of impaired cerebral energy metabolism despite normal perfusion and substrate availability. The underlying pathophysiological mechanisms are not sufficiently elucidated. CMD-glutamate has fewer clinical implications, however, elevated concentrations of this excitatory neurotransmitter are considered to be a marker of ischemia and excitotoxicity. Glycerol is a component of neuronal cell membranes, thus CMD-glycerol concentrations are a surrogate marker of cell membrane damage, e.g., under conditions of hypoxia or ischemia.

MATERIALS AND METHODS

The aim of this review is to summarize the current knowledge of this technique in the critical care management of SAH patients and to discuss its limitations. A MEDLINE search was performed to identify all studies reporting on the clinical use of CMD in aneurysmal SAH patients. The selection process is summarized in **Figure 2**. All identified studies were grouped according to their focus on the brain metabolic changes during the early and subacute phase after SAH, their association with mechanisms of secondary brain injury and outcome.

Definitions

The early phase was defined as the first 72 h after SAH, commonly referred to as "early brain injury" (EBI) (7). Pathological threshold values of parameters commonly given in the SAH literature are CMD-glucose < 0.7 mmol/l (referred to as neuroglucopenia), CMD-lactate > 4 mmol/l, CMD-pyruvate < 120 µmol/l, CMD-glutamate > 10 µmol/l, CMD-glycerol > 50 µmol/l, and CMD-LPR > 40. A CMD-LPR > 40 is referred to as metabolic distress. Recently, the pattern of mitochondrial dysfunction was defined as CMD-LPR > 30 together with CMD-pyruvate levels > 70 µmol/l. Important metabolic profiles in SAH patients are shown in **Table 1**.

RESULTS

The Clinical Use of CMD in the Acute Phase after SAH

The initial phase after SAH is commonly referred to as "early brain injury" and comprises the first 72 h after the bleeding (7), which

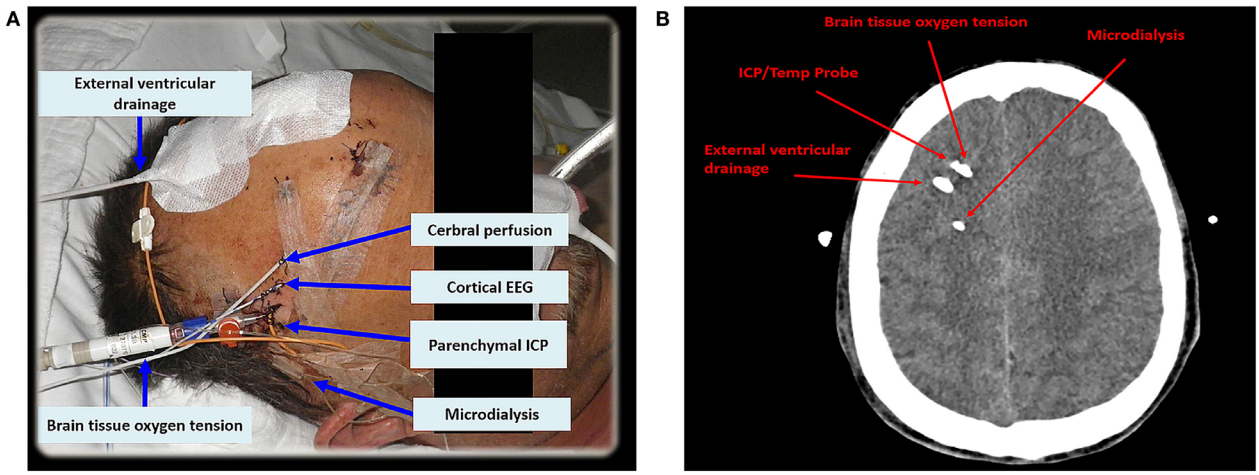

FIGURE 1 | In **(A)**, multimodal neuromonitoring catheters are tunneled in a patient with subarachnoid hemorrhage. **(B)** Shows neuromonitoring catheters placed in the white matter on an axial computed tomography. EEG, electroencephalography; ICP, intracranial pressure; Temp, temperature.

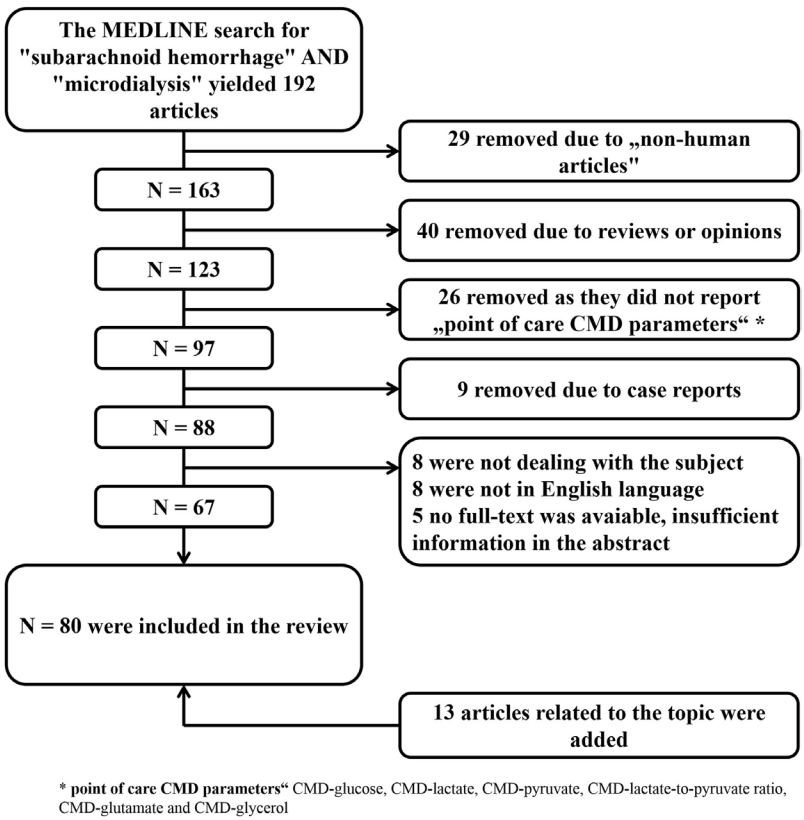

FIGURE 2 | Literature search with selection of articles included in the review. CMD, cerebral microdialysis.

is pathophysiologically related to, but temporally separated from the subsequent occurrence of delayed cerebral ischemia (DCI). EBI is the result of the initial hemorrhage leading to a cascade of ischemic injury, global brain swelling and early mitochondrial dysfunction, as well as pressure-related side effects due to parenchymal hematoma or early hydrocephalus.

Procedural Monitoring

One of the most feared complications early after SAH is aneurysm rebleeding, with the highest risk of occurrence within the first 24 h (8). Importantly, in most studies, metabolic monitoring using CMD started after the aneurysm had been secured (time to monitoring is given in **Tables 3–8**). While no CMD data are

TABLE 1 | Summary of brain metabolic patterns using CMD in SAH patients.

	CMD-glucose	CMD-lactate	CMD-pyruvate	CMD-LPR	CMD-glutamate	CMD-glycerol
Acute focal neurological deficits	↓ to ↓↓	↑↑	n/a	↑↑	↑↑	↑↑, mainly on days 1–2 after subarachnoid hemorrhage
Global cerebral edema	↓ or no difference	↑	↓↓ or ↑ (metabolic distress or hypermetabolism)	↑↑ or ↑	n/a	n/a
Delayed cerebral ischemia	↓↓, decreasing 12–16 h before DCI	↑↑, early, sensitive, but not specific	↓ to ↓↓, rarely independently reported	↑↑, increasing 12–16 h before DCI	↑ to ↑↑, early and sensitive	↑ to ↑↑
Mitochondrial dysfunction	Within normal range	↑↑	Within normal range	↑ to ↑↑	↑ to ↑↑	↑ to ↑↑
Poor outcome	↓↓	↑ to ↑↑, unspecific	↓↓, no increase to normal values	↑↑	↑↑	↑

A single arrow (↑/↓) indicates increased or decreased values compared to normal levels or the control group. Double arrows (↑↑/↓↓) indicate that values are above/below pathologic thresholds (CMD-glucose < 0.7 mmol/l, CMD-lactate > 4 mmol/l, CMD-pyruvate < 120 μmol/l, CMD-LPR > 40, CMD-glutamate > 10 μmol/l, CMD-glycerol > 50 μmol/l). CMD, cerebral microdialysis; n/a, no data available.

available during coiling, several studies have investigated changes in cerebral metabolism during aneurysm surgery (**Table 2**) (9–13). Commonly used CMD sampling intervals between 10 and 60 min seem to be too imprecise to depict periprocedural changes in brain metabolism (9–11), although increases in CMD-LPR and CMD-glutamate levels following prolonged artery clipping and ischemic complications were reported (9, 10). The largest study, including 38 aneurysmal SAH patients, found no significant differences in metabolic markers of brain tissue ischemia during transient artery occlusion (median occlusion time was 14 min) (11). In two patients with a prolonged occlusion time of more than 30 min, a pronounced increase of CMD-glutamate was observed (11). The feasibility of rapid-sampling microdialysis (obtaining values up to every 30 s) was investigated during temporal lobe retraction and transient artery occlusion (13). While metabolic changes reached a maximum after 3–10 min during temporal lobe retraction, increasing CMD-lactate and decreasing CMD-glucose levels were observed until clip removal during artery occlusion (13). A higher sampling frequency in CMD monitoring may help to define a threshold for ischemia to prevent irreversible tissue damage during aneurysm obliteration.

Post-Procedural Monitoring

We identified nine studies focusing on the early phase after aneurysm treatment (**Table 3**) (14–22). In summary, CMD-LPR > 40 during the early phase is a sensitive marker for poor clinical grade on admission (18), radiological evidence of global cerebral edema (15), intracranial hypertension (19), and poor 3-month outcome (14). Critically, low levels of CMD-glucose were associated with acute neurological deficits (16). Trend analysis may indicate the clinical course with "normalization of CMD-parameters" being associated with clinical improvement and pathological evolution of brain metabolism with permanent neurological deficits (16).

CMD and Acute Focal Neurological Deficits after SAH

Most patients underwent aneurysm clipping. In a study including 26 poor-grade SAH patients (68% Hunt and Hess grade IV–V), CMD-LPR and CMD-glutamate were highest at the start of neuromonitoring, indicating metabolic distress (mean CMD-LPR > 40) and excitotoxicity, and significantly decreased thereafter (14). A higher CMD-LPR was associated with poor 3-month outcome (modified Rankin scale 4–6). CMD-glucose significantly decreased, however, did not reach critically low levels in this cohort with systemic glucose levels of 135–150 mg/dl (7.5–8.3 mmol/l) (14). A study including 149 SAH patients, of whom 89 (60%) were admitted with good clinical grades (WFNS grades ≤ 3), reported higher CMD-LPR and CMD-lactate levels in poor-grade patients, already at the start of neuromonitoring, compared to good-grade patients (18). Moreover, higher CMD-glycerol levels were reported in poor-grade patients, significantly decreasing over the first days (18).

In another study including 97 SAH patients, the authors compared patients with and without acute focal neurological deficits immediately after SAH due to SAH-related parenchymal hematoma and/or perioperative/periinterventional ischemia. In patients with focal deficits, CMD-lactate, CMD-LPR, CMD-glutamate, and CMD-glycerol were pathologically increased throughout the first week, whereas these parameters remained within normal range in patients without acute neurological deficits (16, 17). CMD-glucose levels were significantly lower in patients with acute focal deficits (16, 17), despite higher blood glucose concentrations (overall mean blood glucose levels were approximately 7.2–8.3 mmol/l = 130–150 mg/dl) (29). Regarding temporal dynamics, a trend toward normalization of CMD values was associated with clinical improvement, whereas further deterioration was associated with permanent neurological deficits in patients with acute focal deficits (16).

CMD and Admission Global Cerebral Edema

Two studies compared cerebral metabolic changes in patients with and without global cerebral edema (GCE) diagnosed by CT-imaging of the brain. Helbok et al. found an association between a higher frequency of metabolic crisis (significantly higher LPR, lower brain glucose levels) and GCE (15). Zetterling

TABLE 2 | Brain metabolism during aneurysm surgery.

Reference	Study type	Number of patients with SAH	Patient characteristics	Monitoring period	Probe location	Study aim	Main microdialysis findings
(13)	Single-center, prospective, observational	8	WFNS grade I $n = 5$, II $n = 3$	Intraoperative. A CMD catheter was inserted immediately after opening the dura	Territory of the parent artery of the aneurysm	Detecting adverse metabolic events during aneurysm surgery using rapid-sampling microdialysis	During temporal lobe retraction, CMD-lactate levels increased (+0.66 mmol/l) and CMD-glucose levels decreased (−0.12 mmol/l). The peak of these changes was observed after 3–10 min, despite continued retraction. During temporary artery clipping, CMD-lactate levels increased (+0.73 mmol/l) and CMD-glucose levels decreased (−0.14 mmol/l). These changes reached their maximum right before clip removal
(10)	Single-center, prospective, observational	5/12	"Preselected on the basis of anticipated difficulty in surgery"	Intraoperative	Cortical, territory of the parent artery of the aneurysm	Studying amino acid concentrations during periods of cerebral ischemia	CMD-glutamate levels increased between 2.7- and 8.1-fold during ischemic intraoperative complications. No statistical analysis was performed
(9)	Single-center, prospective, observational	10/15	n/a	Intraoperative	Cortical, territory of the parent artery of the aneurysm	To assess metabolic changes during temporary artery clipping	The CMD-LPR ranged from 32 to 65. Clipping <3 min was not followed by an increase in CMD-LPR (42–43). Prolonged clipping was followed by a pronounced increase in CMD-LPR in 2 cases (24–50 and 60–70). No statistical analysis was performed
(11)	Single-center, prospective, observational	38/46	WFNS grade. Poor (III, IV, V) in 18 patients, 7 aneurysms were larger than 25 mm	Intraoperative	Frontal or parietal lobe ipsilateral to the aneurysm	To investigate potential episodes of cerebral ischemia during aneurysm surgery	Temporary artery clipping (median duration 14 min) was not associated with significant changes in brain metabolism. In 2 patients, who post-operatively developed cerebral infarction, clipping for longer than 30 min was associated with a significant CMD-glutamate increase (2–25 μmol/l in 1 patient)
(12) (abstract only)	Single-center, prospective, observational	10/16	"Complex aneurysm surgery"	Intraoperative	n/a	To investigate cerebral metabolic changes during temporary internal carotid artery clipping	Minimal decreases in brain tissue oxygen tension were not associated with metabolic changes, while more pronounced decreases were associated with an increase in CMD-LPR. Prolonged occlusions (42 min) were associated with an increase in CMD-glutamate levels. No statistical analysis was performed

WFNS, world federation of neurological societies; CMD, cerebral microdialysis; n/a, data not available; LPR, lactate-to-pyruvate-ratio.

TABLE 3 | The clinical use of CMD in the acute phase after SAH.

Reference	Study type	Number of patients with SAH	Patient characteristics	Monitoring period	Probe location	Study aim	Main microdialysis findings
(14)	Single-center, prospective, observational	26	Hunt and Hess grade II $n = 2$ (7.7%), III $n = 6$ (23.1%), IV $n = 2$ (7.7%), V $n = 16$ (61.5%)	Monitoring was started 22 h (median) after SAH. Data of the following 144 h are reported	Frontal, ipsilateral to the aneurysm; classified as normal-appearing or perilesional brain tissue	Describing the metabolic profile during the early phase after SAH	Peak levels of CMD-glutamate, CMD-glucose, and the CMD-LPR occurred within the first 24 h of monitoring and decreased over time. CMD-pyruvate concentrations increased compared to baseline values. A higher CMD-LPR was associated with poor outcome. Higher CMD-IL-6 levels were associated with DCI and poor outcome
(15)	Single-center, prospective, observational	39	Hunt and Hess grade I + II $n = 3$ (8%), III $n = 6$ (15%), IV $n = 12$ (31%), V $n = 18$ (46%)	Data are reported for days 2–10 after SAH	Frontal, contralateral to the craniotomy in clipped patients; non-dominant hemisphere in diffuse SAH or ipsilateral in lateralized SAH in coiled patients	Comparing brain metabolism of patients with and without GCE on admission	Patients with GCE showed a higher CMD-LPR and lower CMD-pyruvate and CMD-glucose levels compared to those without. Episodes of CMD-LPR > 40 and metabolic crisis (CMD-LPR > 40 and CMD-glucose < 0.7 mmol/l) were more common in patients with GCE. CMD-LPR > 40 and metabolic crisis were associated with poor outcome
(16)	Single-center, prospective, observational	95	WFNS grade I $n = 40$ (42%), II $n = 11$ (11.5%), III $n = 11$ (11.5%), IV $n = 20$ (21%), V $n = 13$ (14%)	Monitoring was started 34/49 (mean) hours after SAH and maintained for 183/132 (mean) hours in patients with/without acute focal neurological deficits	Vascular territory of the aneurysm; insertion into lesioned tissue was avoided	Investigating brain metabolism in patients with/without acute focal neurological deficits	CMD-glutamate, CMD-glycerol, CMD-lactate concentrations, and the CMD-LPR were higher in patients with acute focal neurological deficits compared to those without. A normalization of values over time was concomitant with an improving clinical condition, further deterioration with permanent neurological deficits
(17)	Single-center, prospective, observational	97	WFNS grade I $n = 37$ (38%), II $n = 13$ (13%), III $n = 9$ (9%), IV $n = 20$ (21%), V $n = 18$ (19%)	Catheters were inserted within 72 h after SAH. Data are reported for days 1–10 after SAH	Vascular territory most likely affected by vasospasm; insertion into lesioned. tissue was avoided	Comparing brain metabolism of patients with acute neurological deficits and DCI to asymptomatic patients	In patients with acute focal neurological deficits, the CMD-glucose concentration was lower, whereas the CMD-lactate, CMD-LPR, CMD-glutamate and CMD-glycerol levels were significantly elevated compared to asymptomatic and DCI patients
(18)	Single-center, prospective, observational	149	WFNS grade 0 $n = 3$ (2%), I $n = 53$ (36%), II $n = 16$ (11%), III $n = 17$ (11%), IV $n = 33$ (22%), V $n = 27$ (18%)	Monitoring was started after aneurysm treatment (mean 24.7 h after SAH) and maintained for 161.8 h (mean)	Vascular territory of the aneurysm; insertion into lesioned tissue was avoided	Investigating the relationship between clinical disease severity, brain metabolism and outcome	The concentrations of all parameters were higher in high-grade (WFNS IV–V) compared to low-grade patients, the differences were significant for CMD-lactate, CMD-LPR and, during the first 2 days, CMD-glycerol
(19)	Single-center, prospective, observational	36	All patients had a WFNS grade of IV or V	Surgery was performed 44/30.7 h after SAH in patients with/without intracranial hypertension. Only patients with complete datasets for the first 7 days were included	Vascular territory of the aneurysm; insertion into lesioned tissue was avoided	To elucidate the impact of intracranial hypertension on brain metabolism	Patients with intracranial hypertension (ICP > 20 mmHg) had significantly lower levels of CMD-glucose and a higher CMD-LPR over the first 7 days after SAH. CMD-glutamate levels were significantly elevated in patients with high ICP on day 1

(*Continued*)

TABLE 3 | Continued

Reference	Study type	Number of patients with SAH	Patient characteristics	Monitoring period	Probe location	Study aim	Main microdialysis findings
(20)	Single-center, prospective, observational	26	Hunt and Hess grade II $n = 1$ (4%), III $n = 7$ (26%), IV $n = 2$ (8%), V $n = 16$ (62%)	Monitoring was started 1 day (median) after SAH and maintained for 4 days (median)	Vascular territory of the aneurysm; classified as normal-appearing or perilesional brain tissue	To investigate the association between neuroinflammation, axonal injury and brain metabolism	High-grade neuroinflammation (CMD-IL-6 levels above median) was associated with CMD-lactate levels > 4 mmol/l, metabolic distress (CMD-LPR > 40), metabolic crisis (CMD-LPR > 40 and CMD-glucose levels < 0.7 mmol/l), DCI and poor functional outcome
(21)	Single-center, prospective, observational	52	WFNS grade I $n = 4$ (8%), II $n = 11$ (21%), III $n = 2$ (4%), IV $n = 24$ (46%), V $n = 11$ (21%)	Monitoring was started 20/28 h (mean) after SAH and maintained for 147/136 h (mean) in patients with/without GCE	Frontal, location in non-injured brain tissue; in 6 patients the CMD probe was located at the craniotomy site	Comparing brain metabolism of patients with and without global cerebral edema on admission	CMD-lactate and CMD-pyruvate levels were significantly, the CMD-LPR non-significantly higher in patients with GCE. There was no difference in CMD-glucose concentrations
(22)	Single-center, prospective, observational	19	Level of consciousness according to the Reaction Level Scale 85 on admission, conscious $n = 11$ (58%), unconscious $n = 8$ (42%)	Monitoring was started 21 h (median) after SAH and maintained for 157 h (median)	Cortical, frontal, in non-injured brain tissue; in 3 patients the CMD probe was located at the craniotomy site	Investigating the association between cerebral metabolites and the level of consciousness on admission	Patients who were unconscious on admission had significantly lower levels of CMD-pyruvate between 96 and 132 h after SAH

SAH, subarachnoid hemorrhage; CMD, cerebral microdialysis; LPR, lactate-to-pyruvate-ratio; IL-6, interleukin-6; GCE, global cerebral edema; WFNS, world federation of neurological societies; DCI, delayed cerebral ischemia; ICP, intracranial pressure.

et al. described a pattern of cerebral hypermetabolism (higher lactate and pyruvate levels, no significant differences in LPR and glucose levels) in GCE patients (21). Despite this discrepancy, these findings indicate altered brain energy metabolism in SAH patients with GCE. Intracranial hypertension (ICP > 20 mmHg), often a result of brain edema or focal lesions, was associated with a pathologically elevated LPR (>40) and significantly lower CMD-glucose levels (19).

The Clinical Use of CMD as a Marker of Cerebral Hypoperfuison and DCI in SAH Patients

Delayed cerebral ischemia occurs in up to 30% of SAH patients, mostly between 4 and 10 days after the hemorrhage, and was defined by an international group of experts as either clinical deterioration or cerebral infarction not attributable to other causes (83). In the literature published before 2010, we found a considerable heterogeneity in the definition of delayed ischemia after SAH. Detailed information on the definition used in individual trials is given in **Table 4**.

Several studies investigated metabolic changes associated with parameters of cerebral perfusion, including CBF, CPP, and imaging surrogates. In summary, a negative correlation between CBF and CMD-lactate, CMD-LPR, CMD-glutamate, and CMD-glycerol has been described, while CMD-pyruvate and CMD-glucose are commonly positively correlated with CBF (25, 31, 32, 35, 38–40). Other studies focused on DCI and found increases in CMD-lactate and CMD-glutamate as early sensitive markers (17). Metabolic derangement with increasing CMD-LPR (> 40) and decreasing CMD-glucose (< 0.7 mmol/l) may occur up to 16 h before DCI onset (26, 32). In the following, we give detailed information on some studies, all studies are listed in **Table 4**.

Schmidt et al. found an association of CPP < 70 mmHg with a higher incidence of metabolic crisis (CMD-LPR > 40 and CMD-glucose levels < 0.7 mmol/l) and further worsening of brain metabolism at lower CPP values (41). Similarly, a higher CMD-LPR and increased episodes of metabolic distress (CMD-LPR > 40) were observed at a CPP < 60 mmHg in another study including 19 SAH patients (23). However, it is important to elaborate that a high CMD-LPR may also indicate metabolic distress in the absence of ischemia. In this regard, Jacobsen et al. defined an elevated LPR (>30), together with pyruvate levels within normal range (>70 μmol/l) as mitochondrial dysfunction and found this pattern to be 7.5-fold more common than metabolic changes indicative for cerebral ischemia (LPR > 30 and CMD-pyruvate < 70 μmol/l) (28). Mitochondrial dysfunction was moreover associated with higher levels of CMD-glucose and lower levels of CMD-glutamate and CMD-glycerol compared to ischemic episodes.

In poor-grade SAH patients requiring sedation and mechanical ventilation, neurological deterioration may not be detected. In these patients, CMD provides useful information on the metabolic state of the injured brain and may even indicate metabolic changes before DCI occurs. Sarrafzadeh et al. reported higher levels of CMD-lactate and CMD-glutamate in patients with DCI compared to those who did not develop DCI already on day 1

TABLE 4 | The clinical use of CMD as a marker of cerebral hypoperfuison and DCI in SAH patients.

Reference	Study type	Number of patients with SAH	Patient characteristics	Monitoring period	Probe location	Definition of ischemia/ DCI	Study aim	Main microdialysis findings
(23)	Single-center, prospective, observational	19	Hunt and Hess grade II $n = 2$ (10%), III $n = 3$ (16%), IV $n = 10$ (53%), V $n = 4$ (21%)	Monitoring was started 1.4 days (mean) after SAH and was maintained for 5.8 days (mean)	Normal-appearing white matter between anterior and middle cerebral artery territory on the side of maximal pathology	Neurological worsening (GCS and NIHSS), persistent $P_{bt}O_2 < 15$ mmHg, flow velocity > 180 cm/s (transcranial Doppler), decreased alpha variability on continuous EEG or reduced blood flow on CT perfusion	To assess CMD-LPR levels with respect to established thresholds of CPP and $P_{bt}O_2$	The CMD-LPR was higher and episodes of CMD-LPR > 40 occurred more often when the CPP was < 60 mmHg. Brain tissue hypoxia was associated with CMD-LPR > 40. About 50% of $P_{bt}O_2$ measurements and 80% of CPP measurements were within normal range when the CMD-LPR was > 40. An LPR > 40 was associated with hospital mortality
(24)	Single-center, prospective, observational	16	Hunt and Hess grade II $n = 13$ (81%), III (19%)	All patients were operated within 1–3 days after SAH	Cortical, frontal or temporal, ipsilateral to the aneurysm	Vasospasm-related clinical disturbances	Associating CMD findings with impending ischemia	Patients with DCI showed increasing levels of CMD-lactate and decreasing levels of CMD-glucose. CMD-glutamate levels were increased in the proximity of infarcts on head CT
(25)	Single-center, prospective, observational	6	Hunt and Hess grade II $n = 2$ (33.3%), III $n = 2$ (33.3%), IV $n = 2$ (33.3%)	n/a	Cortical, frontal	By PET: regional oxygen extraction ratio > 125% and $CMRO_2 \geq 45\%$ of the corresponding contralateral region or $CMRO_2 < 45\%$ of the corresponding contralateral region	To associate brain metabolite concentrations with ischemia detected by PET	Ischemia, defined using the cerebral metabolic rate of oxygen and oxygen extraction ratio detected by PET, was concomitant with high levels of CMD-lactate, CMD-glutamate, and CMD-LPR. No statistical analysis was performed
(26)	Single-center, prospective observational	32	Hunt and Hess grade I + II $n = 3$ (9%), III $n = 6$ (19%), IV $n = 9$ (28%), V $n = 14$ (44%)	CMD data are reported in relation to head CT scans between 1 and 10 days after the hemorrhage	White matter; frontal, contralateral to the craniotomy in clipped patients; non-dominant hemisphere in diffuse SAH or ipsilateral in lateralized SAH in coiled patients	Clinical symptoms or new infarcts on CT or MRI attributable to vasospasm	Comparing the metabolic patterns preceding head CT scans with and without new infarcts	CMD-lactate levels and the CMD-LPR significantly increased and CMD-glucose concentrations significantly decreased (to 0.5 mmol/l) when new infarcts were detected on a head CT scan. This was not observed in contralateral (from the CMD probe) or distant (>4 cm) infarction. Metabolic crisis (CMD-LPR > 40 and CMD-glucose < 0.7 mmol/l) was more common when new infarcts were revealed
(27)	Single-center, prospective observational	4	Hunt and Hess grade II $n = 2$ (50%), III $n = 1$ (25%), IV $n = 1$ (25%)	CMD was started between 12 and 28 h after SAH and maintained until about 200 h after SAH	Cortical, frontal	Neurological signs and neuro-imaging (CT/PET)	Associating the temporal dynamics of cerebral CMD-glycerol levels with ischemic events	Ischemic events were associated with a pronounced increase in CMD-glycerol levels (descriptive). In 1 patient without ischemia CMD-glycerol remained low after the peak immediately at the start of monitoring. CMD-glycerol levels correlated with CMD-LPR, CMD-glutamate, and CMD-lactate concentrations

(*Continued*)

TABLE 4 | Continued

Reference	Study type	Number of patients with SAH	Patient characteristics	Monitoring period	Probe location	Definition of ischemia/ DCI	Study aim	Main microdialysis findings
(28)	Single-center, prospective, observational	55	Admission GCS 15 *n* = 9 (16%), 9–14 *n* = 20 (36%), <9 *n* = 26 (47%)	n/a	Craniotomy site in patients undergoing open surgery; frontal in patients not undergoing open surgery	Biochemical: CMD-LPR > 30 and CMD-pyruvate < 70 μmol/l	Proposing a metabolic pattern suggestive for mitochondrial dysfunction	The pattern of mitochondrial dysfunction (LPR > 30 and pyruvate > 70 μmol/l) was more common (7.5-fold) than the pattern of ischemia (LPR > 30 and pyruvate < 70 μmol/l) and associated with higher levels of glucose and lower levels of glutamate and glycerol
(29)	Single-center, prospective, observational	170	WFNS grade 0 *n* = 3 (2%), I *n* = 58 (34%), II *n* = 22 (13%), III *n* = 18 (11%), IV *n* = 38 (22%), V *n* = 31 (18%)	Data are reported for days 1–7 after SAH	Vascular territory of the aneurysm; insertion into lesioned tissue was avoided	Symptomatic vasospasm	To compare systemic and CMD-glucose levels with respect to acute focal neurological deficits and DCI	Patients with acute neurological deficits and patients developing DCI had higher blood glucose levels on admission and over the first 7 days compared to asymptomatic patients, but significantly lower CMD-glucose levels. The CMD-LPR was highest in patients with acute neurological deficits, followed by DCI patients and asymptomatic patients
(30)	Single-center, prospective observational	10	Hunt and Hess grade II *n* = 2 (20%), III *n* = 7 (70%), IV *n* = 1 (10%)	Catheters were inserted 1 day (median) after SAH, CMD was performed for 4–11 days (median)	Cortical, frontal or temporal, ipsilateral to the aneurysm	Clinical deterioration associated with cerebral vasospasm	Introducing a bedside analyzer. Associating metabolic patterns with ischemia	DCI was associated with high levels of CMD-lactate, CMD-glutamate, CMD-glycerol, and CMD-LPR and low concentrations of CMD-glucose. Assumed "normal" ranges of metabolites: 1–4 mmol/l (CMD-glucose), 1–3 mmol/l (CMD-lactate), 10–50 μmol/l (CMD-glycerol), 2–10 μmol/l (CMD-glutamate), 10–40 (CMD-LPR)
(31)	Single-center, retrospective, observational	21	Modified Fisher scale III *n* = 6 (29%), IV *n* = 15 (71%)	Time point of monitoring start not described. Monitoring lasted 10 days in all patients	Frontal, ipsilateral to the most prominent pathology, intact brain tissue	n/a	Correlating the concentrations of cerebral metabolites with CBF	There was a positive correlation of CBF (measured by a thermo-dilution probe) with CMD-pyruvate and CMD-glucose levels and a negative correlation with CMD-lactate and CMD-glycerol levels and the CMD-LPR
(32)	Single-center, prospective observational	20	WFNS grade II *n* = 6 (30%), IV *n* = 2 (10%), V *n* = 12 (60%)	CMD sampling was started 14 h (median) after SAH and maintained for 8 days (median)	White matter, frontal watershed of the non-dominant hemisphere, visually normal brain	CBF < 32.5 ml/100 g/min and mean transit time > 5.7 s in CT perfusion	Comparing metabolic profiles between patients with and without cerebral hypoperfusion measured by CT	The critical perfusion threshold was defined as CBF < 32.5 ml/100 g/min and mean transit time > 5.7 s. Patients with hypoperfusion had a significantly higher CMD-LPR (51 vs. 31) and lower CMD-glucose levels (0.64 vs. 1.22 mmol/l). During the 18 h before the perfusion CT was performed, there was a significant increase in CMD-LPR and decrease in CMD-glucose levels in the hypoperfusion group, but not in patients with normal CBF

(*Continued*)

TABLE 4 | Continued

Reference	Study type	Number of patients with SAH	Patient characteristics	Monitoring period	Probe location	Definition of ischemia/ DCI	Study aim	Main microdialysis findings
(33)	Single-center, prospective observational	10	Hunt and Hess grade II *n* = 3 (30%), III *n* = 4 (40%), IV *n* = 3 (30%)	Start time is not exactly given (figures indicate 12–30 h after SAH). Monitoring lasted 6–11 days (range)	Cortical, frontal	CT findings and clinical course	To match CMD data with CT findings, clinical course and outcome	CMD-lactate elevations were frequently observed without obvious cause, while CMD-LPR reflected ischemia and, during days 0–4, correlated with outcome. In an infracted area, CMD-glucose levels fell to and remained at 0. Zero-levels of CMD-glucose were observed more frequently in patients with poor outcome. Patients with poor outcome had significantly higher CMD-glutamate levels
(34)	Single-center, prospective, observational	18	WFNS grade I *n* = 4 (22%), II *n* = 4 (22%), III *n* = 1 (6%), IV *n* = 6 (33%), V *n* = 2 (17%)	CMD monitoring started within 24 h after admission. Data were analyzed on days 1–12 after SAH	Vascular territory of the aneurysm	New focal neurological impairment or decrease ≥ 2 points in GCS score for at least 1 h, not attributable to other causes	Investigating an association between early onset pneumonia and cerebral metabolism	Elevated lactate levels on day 7 were associated with DCI
(35)	Single-center, prospective, observational	9	Hunt and Hess grade I *n* = 2 (22.2%), II *n* = 2 (22.2%), III *n* = 2 (22.2%), IV *n* = 2 (22.2%), V *n* = 1 (11.1%)	Within 72 h of admission	Cortical, right frontal lobe	Neurologic deficit or deterioration that could not be explained by other reasons	Associating the concentrations of cerebral metabolites with CBF	Lower CBF, measured by Xenon-CT, occurred together with higher levels of CMD-glutamate and a higher CMD-LPR. There was a descriptive association between CMD-LPR > 25 and a CBF < 22 ml/100 g/min. No statistical analyses were performed
(36)	Single-center, prospective, observational	78	WFNS grade I *n* = 26 (33%), II *n* = 11 (14), III *n* = 9 (12%), IV *n* = 18 (23%), V *n* = 14 (18%)	Monitoring was started 46 h (mean) after SAH and maintained for 155 h (mean)	White matter, vascular territory most likely affected by vasospasm	Insidious onset of confusion or appearance of a focal neurological deficit	To assess the sensitivity and specificity of CMD for confirming DCI	Baseline values did not differ between patients with and without DCI. Threshold values were set at CMD-lactate > 4 mmol/l and CMD-glutamate > 3 μmol/l. CMD showed a higher specificity for confirming DCI than conventional angiography and TCD
(37)	Single-center, prospective observational	33	WFNS grade 3.5 (median), 1–5 (range)	Monitoring was started 29.5 h (mean) after SAH and maintained for 112 h (mean)	Cortical, frontal or temporal, visually non-injured tissue	Decrease in the level of consciousness (≥1 step in the RLS score) or new focal neurological deficit, not due to other causes but vasospasm	Identifying a metabolic pattern indicative of ischemia	Five hours of CMD-LPR > 40 during a 10-h period were defined as ischemic pattern. 12 episodes of this pattern occurred, of which 5 were attributable to early infarcts and 6 to DCI. Only 6 of 15 cases of DCI were associated with this pattern, which, in these cases, occurred 16.7 h before DCI

(*Continued*)

TABLE 4 | Continued

Reference	Study type	Number of patients with SAH	Patient characteristics	Monitoring period	Probe location	Definition of ischemia/ DCI	Study aim	Main microdialysis findings
(38)	Single-center, prospective, observational	7	Symptoms on admission, 3 patients (43%) were described as asymptomatic, 4 (57%) suffered from either aphasia or hemiparesis	Clipping and CMD probe insertion were performed within 24 h after SAH. The mean monitoring time was 8.5 days	Vascular territory of the aneurysm; insertion in lesioned tissue was avoided	New focal neurological signs or deterioration in level of consciousness, excluding other causes but vasospasm	To associate PET findings indicative of hypoxia with CBF and cerebral metabolism	In regions with ^{18}F-FMISO uptake, a PET marker of hypoxia, CMD-glutamate levels were significantly higher compared to regions without uptake. No differences in energy metabolite concentrations were observed
(39)	Single-center, prospective observational	15	Neurological symptoms, 5 patients (33.3%) were classified as asymptomatic. 10 patients (66.6%) suffered either from aphasia (1), frontal lobe dysfunction (2), paresis (5) or coma (2)	Monitoring was started 52.8 h (mean) after SAH and maintained for 201/211 h (mean) in patients with/ without symptoms of ischemia	Brain parenchyma most likely affected by vasospasm	Neurological deficits	To associate brain metabolite concentrations with CBF and ischemia measured by PET	On the day of PET, levels of CMD-lactate, CMD-glutamate, CMD-glycerol and the CMD-LPR were significantly higher in symptomatic patients. There were strong inverse correlations between CBF (measured by PET) and CMD-glutamate and CMD-glycerol levels
(40)	Single-center, prospective observational	13	WFNS grade I $n = 3$ (23%), II $n = 2$ (15.5%), III $n = 4$ (33%), IV $n = 3$ (23%), V $n = 1$ (15.5%)	Monitoring was started 52.8 h (mean) after SAH and maintained for 201 h (mean)	White matter, vascular territory most likely affected by vasospasm; insertion in lesioned tissue was avoided	Worsening of headache or focal neurological deficits not present at admission, between 2 and 14 days after SAH, not attributable to other causes	Associating CMD parameters with symptoms of ischemia and CBF	3-day medians were compared between symptomatic (ischemic) and asymptomatic intervals. The CMD-LPR, CMD-lactate, and CMD-glutamate levels were higher during symptomatic intervals. There were strong inverse correlations between CBF (measured by PET) and CMD-glutamate and CMD-glycerol levels. CMD-lactate levels > 4 mmol/l were an indicator of critically low CBF (< 20 ml/100 g/min)
(17)	Single-center, prospective, observational	97	WFNS grade I $n = 37$ (38%), II $n = 13$ (13%), III $n = 9$ (9%), IV $n = 20$ (21%), V $n = 18$ (19%)	Catheters were inserted within 72 h after SAH. Data are reported for days 1–10 after SAH	Vascular territory most likely affected by vasospasm; insertion into lesioned tissue was avoided	Worsening of headache, stiff neck, insidious onset of confusion, disorientation or drowsiness, or focal neurological deficits, between 2 and 14 days after SAH, not attributable to other causes	Comparing brain metabolism of patients with acute neurological deficits and DCI to asymptomatic patients	DCI patients had higher lactate and glutamate concentrations on days 1–8 and a higher LPR on days 3–8 compared with asymptomatic patients
(41)	Single-center, prospective, observational	30	Hunt and Hess grade II + III $n = 5$ (17%), IV $n = 10$ (33%), V $n = 15$ (50%)	Monitoring was started 3 days (median) after SAH and maintained for 110 h (median)	Frontal, ipsilateral to lateralized aneurysms, right frontal lobe in case of midline aneurysms	Clinical deterioration or cerebral infarction attributable to vasospasm	To identify the relation between CPP thresholds and brain metabolic crisis	Metabolic crisis (CMD-LPR > 40 and CMD-glucose levels ≤ 0.7 mmol/l) was associated with a CPP < 70 mmHg, Hunt and Hess grade 5, intraventricular or parenchymal hemorrhage, hydrocephalus, ICP > 20 mmHg and serum glucose levels < 6.6 mmol/l. Metabolic crisis was associated with poor outcome

(*Continued*)

TABLE 4 | Continued

Reference	Study type	Number of patients with SAH	Patient characteristics	Monitoring period	Probe location	Definition of ischemia/ DCI	Study aim	Main microdialysis findings
(42)	Single-center, prospective observational	18	Hunt and Hess grade I *n* = 4 (22%), II *n* = 4 (22%), III *n* = 7 (39%), IV *n* = 3 (17%)	Time point of monitoring start not described. Monitoring was maintained up to 7 days after SAH	Subcortical, either radiologically normal-appearing brain tissue or ischemic tissue indicated by brain CT	Infarction on cerebral CT scans	Comparing brain metabolism between patients without ischemia and patients suffering brain death	In patients without evidence of cerebral ischemia CMD-glucose and CMD-pyruvate levels were significantly higher and CMD-glutamate, CMD-glycerol, and CMD-lactate levels and the CMD-LPR were lower compared to patients becoming brain dead. During the time between brain death (complete ischemia) and cessation of treatment, CMD-glucose, and CMD-pyruvate were not detectable and there was a further increase of CMD-glutamate and CMD-glycerol levels
(43)	Single-center, prospective observational	42	WFNS grade I *n* = 13 (31%), II *n* = 10 (24%), III *n* = 3 (7%), IV *n* = 13 (31%), V *n* = 3 (7%)	Time point of monitoring start not described. CMD was performed for 5 days (mean)	Cortical and white matter, vascular territory of the parent vessel of the aneurysm	Diagnosed by the neurosurgeon on call	Assessing the predictive value of a CMD pattern for DCI	The pattern was defined as an increase in CMD-LPR and lactate-to-glucose-ratio > 20%, followed by an increase in CMD-glycerol levels > 20%. In 17 of 18 patients, in whom DCI occurred, the pattern was found. It preceded the event by 11 h (glycerol peak to DCI). The ischemic pattern occurred in 3 patients without DCI
(44)	Single-center, prospective observational	60	WFNS grade I *n* = 20 (33%), II *n* = 9 (15%), III *n* = 5 (8%), IV *n* = 15 (25%), V *n* = 11 (18%)	Monitoring was started after clipping, 28 h (mean) after SAH. Monitoring was maintained for 174 h (mean)	White matter, vascular territory most likely affected by vasospasm; insertion into lesioned tissue was avoided	Symptomatic vasospasm defined as insidious onset of confusion or focal neurological deficit	To assess the predictive ability of CMD regarding DCI compared to TCD and conventional angiography	Baseline values (first 72 h) did not differ between DCI and non-DCI patients. In DCI patients, CMD-glucose levels decreased (64%) and CMD-lactate and CMD-glutamate levels increased (112 and 400%) thereafter. The pathological threshold was defined as CMD-lactate levels > 4 mmol/l and CMD-glutamate levels > 3 μmol/l for 6 consecutive hours. Using this pattern, CMD showed a higher specificity than TCD and angiography

SAH, subarachnoid hemorrhage; CMD, cerebral microdialysis; LPR, lactate-to-pyruvate-ratio; CPP, cerebral perfusion pressure; $P_{bt}O_2$, brain tissue oxygen tension; n/a, data not available; PET, positron emission tomography; WFNS, world federation of neurological societies; DCI, delayed cerebral ischemia; CBF, cerebral blood flow; FMISO, fluoromisonidazole; TCD, transcranial Doppler; CT, computed tomography; GCS, Glasgow coma scale; EEG, electroencephalography; MRI, magnetic resonance imaging; NIHSS, National Institutes of Health Stroke Scale; $CMRO_2$, cerebral metabolic rate of oxygen; RLS, reaction level scale.

and throughout the first week after the bleeding (17). A higher CMD-LPR was observed from day 3 on (17). This is in line with findings by Nilsson et al., who concluded that CMD-lactate and CMD-glutamate may be the earliest and most sensitive markers of ischemia, followed by the CMD-LPR and CMD-glycerol (30). Furthermore, lower brain glucose levels during the first 3 days after SAH were reported in DCI patients, despite higher blood glucose concentrations when compared to patients who did not develop DCI (29).

Changes in CMD parameters were observed even 12–16 h before the occurrence of DCI and included a significant increase in CMD-LPR to values >40 and decreases in CMD-glucose to levels <0.7 mmol/l (26, 32, 37). At a comparable time point, also elevations of CMD-lactate, CMD-glutamate, and CMD-glycerol were reported (43, 44). The sensitivity of the method is limited due to the local measurement. Therefore, ischemia occurring in brain tissue distant to the monitoring catheter may not be detected (26, 37). Lack of specificity results from metabolic distress secondary to non-ischemic CMD-LPR elevations and hyperglycolytic (non-ischemic) lactate increase (28, 72). Nevertheless, CMD had a higher specificity for predicting DCI than transcranial ultrasound and conventional angiography (44). Unfortunately, most studies do not report on the effect of current treatment strategies for DCI (induced hypertension, intraarterial spasmolysis). Sarrafzadeh et al. reported a decrease in CMD-glutamate levels, but no change in other parameters, associated with "triple-H therapy" (17). Further research focusing on metabolic changes following such interventions is surely warranted.

In summary, studies assessing CBF directly in the region around the CMD probe revealed a highly consistent metabolic pattern of increased CMD-lactate, CMD-glutamate, CMD-glycerol levels, and CMD-LPR during episodes of hypoperfusion, whereas CMD-glucose and CMD-pyruvate levels were positively correlated with CBF. CMD-lactate and CMD-glutamate are early and sensitive markers of impending DCI, but lack of specificity. However, CMD parameters showed a higher specificity for predicting DCI than transcranial ultrasound and conventional angiography (37, 43, 44). Regarding trend analyses, a CMD-LPR increase above 40 and decreasing CMD-glucose concentrations preceded the occurrence of delayed ischemia by several hours (26, 32); however, ischemia that occurred remote from the CMD probe or in the contralateral hemisphere was not detected (26).

CMD in Monitoring Treatment Effects

Several studies have investigated the effect of pharmacological and non-pharmacological interventions on cerebral metabolism as summarized in **Table 5**. Studied interventions included the treatment of intracranial hypertension, either with osmotherapy (45, 46), by cerebrospinal fluid drainage *via* external ventriculostomy (53), or by decompressive craniectomy (49, 50), fever treatment with diclofenac or targeted temperature management (51, 52, 54), the management of anemia and administration of packed red blood cells (47, 48, 52), and the administration of erythropoietin or verapamil (55, 56). The impact of enteral nutrition and insulin on brain metabolism will be discussed in the next chapter.

CMD and Systemic Glucose Management

There is still an ongoing debate on the optimal systemic glucose target in critically ill patients (84, 85). CMD-glucose levels represent the net effect of delivered glucose and glucose consumption. Little is known about the impact of glucose transport and diffusion in acutely brain injured patients.

Severe hyperglycemia (>200 mg/dl = 11.1 mmol/l) is associated with poor outcome in SAH patients (86). Some studies investigated the association between systemic and brain interstitial glucose levels (**Table 6**). Oddo et al. described the brain metabolic profile during episodes of "low" (<4.4 mmol/), "tight" (4.4–6.7 mmol/l), "intermediate" (6.8–10 mmol/l), and high blood glucose levels in a mixed population of neurocritical care patients, including 10 patients with SAH. Compared to intermediate systemic glucose levels, tight glycemic control was associated with lower CMD-glucose levels and more episodes of CMD-glucose < 0.7 mmol/l, as well as a higher CMD-LPR and more episodes of metabolic crisis (CMD-LPR > 40 and CMD-glucose concentrations < 0.7mmol/l). Metabolic crisis and CMD-glucose < 0.7 mmol/l were associated with higher hospital mortality (61). The significant association of systemic- and CMD-glucose is supported by the results of other groups (59, 66); however, some studies indicate a poor correlation (63, 67), especially in the injured brain. Decreased CMD-glucose is moreover observed when delivery is reduced (i.e., reduction in CBF) or under conditions of increased consumption (i.e., higher body temperature, seizures and the occurrence of cortical spreading depolarizations) (66, 78, 82).

Pathologically low CMD-glucose levels (<0.7 or <0.6 mmol/l) were associated with poor outcome in SAH patients (41, 61, 63). In the recent consensus statement on the use of CMD, clinical experts suggest to intervene when pathologically low CMD-glucose levels are detected (2). This attempt should only be made with respect to probe location (in healthy-appearing brain tissue on head computed tomography), baseline blood glucose levels, and in the absence of brain ischemia. Proposed interventions targeting higher systemic glucose levels include intravenous or enteral glucose administration and the reevaluation of insulin treatment (2).

While no data on intravenous glucose administration are available, three studies sought to investigate the impact of enteral feeding on cerebral metabolism. Schmidt et al. did not observe a direct relation between CMD-glucose levels and the energy content of the administered enteral nutrition (66). Other groups investigating the temporal association of enteral feeding and the metabolic profile revealed time-related increases in CMD-glucose levels not affecting other CMD parameters (58, 59). CMD-glucose levels even increased from critically low (<0.7 mmol/l) levels at baseline independent of probe location (59).

Insulin treatment was associated with a decrease of CMD-glucose independent of serum glucose levels, resulting in a higher incidence of critically low CMD-glucose levels (<0.6 mmol/l) (64, 67), especially under conditions of cerebral metabolic distress (LPR > 40) (66). Moreover, rapid reductions in serum glucose concentrations were associated with a decrease in CMD-glucose (57). In line with this, a higher serum glucose variability was associated with a higher rate of cerebral metabolic distress (60). In

TABLE 5 | CMD in monitoring treatment effects in SAH patients.

Reference	Study type	Number of patients with SAH	Patient characteristics	Monitoring period	Probe location	Study aim	Main microdialysis findings
(45)	Single-center, prospective, interventional	14	n/a	n/a	Frontal or parietal lobe ipsilateral to the aneurysm	To assess the impact of hypertonic saline on cerebral perfusion and metabolism	30 and 60 min after the infusion of hypertonic saline, the CMD-LPR decreased in 9 of 14 patients. Overall, this effect was not significant
(46)	Single-center, prospective, observational	9/12	Hunt and Hess grade 5 (median), 4–5 (interquartile range)	Monitoring was initiated at day 2 (median) after SAH and maintained for 8 days (median)	White matter, frontal, hemisphere deemed at greatest risk for secondary injury	To assess the impact of intravenous mannitol on cerebral metabolism	Mannitol was administered due to an ICP crisis > 20 mmHg. The highest CMD-LPR was recorded at the time point of the start of the infusion (mean of 47). The CMD-LPR significantly decreased by 20% over 2 h, CMD-lactate and CMD-pyruvate levels decreased non-significantly, CMD-glucose remained unaffected
(47)	Single-center, retrospective, observational	34	Hunt and Hess grade III $n = 6$ (18%), IV $n = 12$ (35%), V $n = 16$ (47%)	n/a	White matter, frontal, hemisphere deemed at greatest risk for secondary injury	Comparing the frequencies of metabolic distress between different hemoglobin levels	Compared to hemoglobin concentrations between 10 and 11 g/dl, episodes of CMD-LPR > 40 occurred 1.9 times more often when hemoglobin was between 9 and 10 g/dl, and 3.8 times more often when hemoglobin was below 9 g/dl (45% of measurements showed CMD-LPR > 40, respectively)
(48)	Single-center, prospective, observational	15	Hunt and Hess grade IV + V in 80% of patients	n/a	White matter, hemisphere deemed at greatest risk for secondary injury or right frontal lobe; visually normal tissue	Investigating the impact of packed red blood cell infusions on brain metabolism	Over a 12-h period after a packed red blood cell infusion, no significant changes in cerebral metabolism were observed, despite an increase in CPP and $P_{bt}O_2$
(49)	Single-center, prospective, observational	18	WFNS grade I $n = 3$ (17%), III $n = 2$ (11%), IV $n = 4$ (22%), V $n = 9$ (50%)	Catheters were inserted 15/12 h (median) after SAH and CMD was maintained for 164/180 h (median) in patients with/without craniectomy	Vascular territory of the aneurysm; insertion into lesioned tissue was avoided	To assess the impact of decompressive craniectomy (due to refractory intracranial hypertension) on brain metabolism	Compared to a control group with normal ICP, patients with intracranial hypertension (ICP < 20 mmHg for > 6 h) had lower levels of CMD-glucose and higher levels of CMD-lactate, CMD-glutamate, CMD-glycerol, and CMD-LPR. Concentrations of CMD-glucose and CMD-pyruvate were higher and levels of CMD-glycerol were lower in patients who underwent decompressive craniectomy compared to those who were treated conservatively. The metabolic pattern of CMD-LPR > 25, CMD-glycerol > 80 μmol/l and CMD-glutamate > 10 μmol/l for > 6 h preceded the onset of refractory intracranial hypertension by 40 h (median)
(50)	Single-center, prospective, observational	182	WFNS grade 0 $n = 3$ (2%), I $n = 61$ (37%), II $n = 23$ (14%), III $n = 17$ (10%), IV $n = 35$ (21%), V $n = 25$ (15%)	Monitoring was started immediately after aneurysm treatment (23/13 h after SAH, mean) and maintained for 169/172 h (mean) in patients with/ without ICP intracranial hypertension	Vascular territory of the aneurysm; insertion into lesioned tissue was avoided	Investigating the impact of intracranial hypertension on cerebral metabolism	Higher CMD-LPR, CMD-glutamate, and CMD-glycerol levels and lower CMD-glucose levels were observed in patients with ICP > 20 mmHg. A metabolic pattern of LPR > 25, glutamate > 10 μmol/l and glycerol > 80 μmol/l preceded the first ICP increase > 20 mmHg. Decompressive craniectomy was associated with a decrease in CMD-glycerol and an increase in CMD-glutamate levels. Higher CMD-LPR and CMD-glutamate levels were associated with poor outcome

(*Continued*)

TABLE 5 | Continued

Reference	Study type	Number of patients with SAH	Patient characteristics	Monitoring period	Probe location	Study aim	Main microdialysis findings
(51)	Single-center, prospective, observational	18	Hunt and Hess grade II *n* = 2 (11%), III *n* = 4 (22%), IV *n* = 8 (45%), V *n* = 4 (22%)	Monitoring was initiated 1 day (median) after SAH. Between 37 and 168 hourly samples were obtained per patient	White matter, contralateral to the maximal focal injury, normal-appearing tissue	To assess the impact of induced normothermia on cerebral metabolism	When normothermia (37°C) was induced due to refractory fever (≥38.3°C), it was associated with a decrease in CMD-LPR and fewer episodes of metabolic distress (CMD-LPR > 40). Patients with poor outcome had a higher CMD-LPR
(52)	Single-center, prospective, observational	20	Hunt and Hess grade II *n* = 2 (10%), III *n* = 4 (20%), IV *n* = 10 (50%), V *n* = 4 (20%)	Monitoring was started with 48 h after SAH and maintained for 7 days (median)	White matter, vascular territory most likely affected by vasospasm; radiologically normal-appearing tissue	To associate hemoglobin concentrations with cerebral metabolism	Hemoglobin concentrations < 9 g/dl were associated with a higher absolute CMD-LPR and more episodes of LPR > 40 compared to higher hemoglobin levels
(53)	Single-center, prospective, observational	33	WFNS grade 3 (median), 1–5 (range)	CMD sampling was started 29.5 h (mean) after SAH and maintained for 112 h (mean)	Cortical, frontal or temporal, non-injured brain tissue	To assess the relationship between ICP, CPP and cerebral metabolism	CPP was positively correlated with CMD-pyruvate levels. Episodes of ICP > 10 mmHg were associated with lower levels of CMD-pyruvate and higher levels of CMD-lactate, CMD-pyruvate, and CMD-LPR. In 3 patients, opening the ventricular drain was associated with increasing CMD-pyruvate levels (descriptive)
(54)	Single-center, prospective, observational	21	Hunt and Hess grade II + III *n* = 6 (29%), IV + V *n* = 13 (71%)	Monitoring was initiated at day 1 (median) after SAH and maintained for 12 (median) days	White matter, hemisphere deemed at greatest risk for secondary injury	To assess the impact of intravenous diclofenac on CPP, $P_{bt}O_2$ and cerebral metabolism	Despite a decrease of body temperature, CPP and $P_{bt}O_2$, no changes in cerebral metabolism were observed
(55)	Single-center, prospective, randomized, controlled, double-blind	54 (35 with CMD)	WFNS grade I *n* = 22 (41%), II *n* = 5 (9%), III *n* = 1 (2%), IV *n* = 16 (30%), V *n* = 10 (18%)	Data are reported for days 1–14 after SAH	Vascular territory of the aneurysm	To study the efficacy and safety of EPO in SAH patients	The administration of EPO was associated with higher CMD-glycerol levels. No differences in other CMD parameters were observed
(56)	Single-center, prospective, observational	11	Hunt and Hess grade III *n* = 3 (27%), IV *n* = 2 (18%), V *n* = 6 (55%)	Measurements were performed between after 4–14 days (range) after SAH	White matter, frontal watershed, ipsilateral to the aneurysm or contralateral to the craniotomy in clipped patients	To investigate the impact of intraarterial verapamil on brain metabolism	There was a significant increase in CMD-glucose levels 9 h after the administration of intraarterial verapamil (1.2–1.53 mmol/l). No significant changes in other CMD parameters were observed

n/a, data not available; CMD, cerebral microdialysis; LPR, lactate-to-pyruvate-ratio; SAH, subarachnoid hemorrhage; CPP, cerebral perfusion pressure; $P_{bt}O_2$, brain tissue oxygen tension; ICP, intracranial pressure; EPO, erythropoietin.

TABLE 6 | CMD and systemic glucose management in SAH patients.

Reference	Study type	Number of patients with SAH	Patient characteristics	Monitoring period	Probe location	Study aim	Main microdialysis findings
(57)	Single ceter, prospective, observational	28	Hunt and Hess grade III $n = 6$ (21%), IV $n = 8$ (29%), V $n = 14$ (50%)	Monitoring was initiated on day 2 (median) after SAH and maintained for 6 days (median)	White matter, frontal, tissue at risk or contralateral to the craniotomy	To assess the relationship between rapid reductions in serum glucose and brain metabolism	Reductions in serum glucose by 25% (within normal range) were independently associated with consecutive metabolic crisis (CMD-LPR > 40 and CMD-glucose < 0.7) and increasing CMD-LPR. There was a concomitant decrease in CMD-glucose and CMD-pyruvate. A higher CMD-LPR was associated with hospital mortality
(58)	Single ceter, prospective, observational	12	Glasgow coma scale score 5–7 (range)	Measurements took place between 1 and 5 days after SAH	Cortical, right frontal lobe	To investigate the impact of enteral nutrition on cerebral metabolism	CMD-glucose levels significantly increased following the first bolus of enteral nutrition (2.5–3.7 mmol/l). No changes in CMD-lactate, CMD-pyruvate, CMD-glutamate, or CMD-glycerol were observed. No insulin was used during the measurements
(59)	Single ceter, prospective, observational	17	Hunt and Hess grade II $n = 2$ (12%), III $n = 6$ (35%), IV $n = 2$ (12%), V $n = 7$ (41%)	Measurements took place between 3 and 22 days after SAH	White matter, hemisphere deemed at greatest risk for secondary injury, classified as normal-appearing or perilesional brain tissue	To investigate the impact of enteral nutrition on cerebral glucose levels	Enteral nutrition significantly increased CMD-glucose levels (1.59–2.03 mmol/l) with a delay of 3 h. This increase was independent of insulin administration, absolute levels of serum glucose, evidence of cerebral metabolic distress, and microdialysis probe location. Also critically low CMD-glucose concentrations were increased. There was a significant association between serum and CMD-glucose levels
(60)	Single ceter, prospective, observational	28	Hunt and Hess grade II $n = 1$ (4%), III $n = 5$ (18%), IV $n = 8$ (29%), V $n = 14$ (50%)	Monitoring was started 2 days (median) after SAH and maintained for 6 days (median)	White matter, frontal, hemisphere deemed at greatest risk for secondary injury	To investigate the impact of blood glucose variability on cerebral metabolism	A higher systemic glucose variability, defined as the SD of measured concentrations per day, was associated with a higher risk of developing at least one episode of CMD-LPR > 40 per day
(61)	Single ceter, prospective, observational	10/20	GCS score 7 (median), 3–10 (range)	CMD monitoring was started 45 h (median) after SAH and maintained for 96 h (median)	Frontal, near the area of lesioned tissue or right frontal lobe in patients with diffuse injury	Investigating the impact of tight glycemic control on cerebral metabolism	Blood glucose levels were defined as "tight" (4.4–6.7 mmol/l) or "intermediate" (6.8–10 mmol/l). Tight blood glucose was associated with lower levels of CMD-glucose, more episodes of CMD-glucose < 0.7 mmol/l, higher CMD-LPR and a more frequent occurrence of metabolic crisis (CMD-LPR > 40 and CMD-glucose levels < 0.7 mmol/l). Low CMD-glucose levels and metabolic crisis were associated with higher hospital mortality
(62)	Single ceter, prospective, observational	178	WFNS grade I $n = 65$ (37%), II $n = 22$ (12%), III $n = 20$ (11%), IV $n = 36$ (20%), V $n = 35$ (20%)	CMD was performed on days 1–7 after SAH	Vascular territory of the aneurysm; insertion into lesioned tissue was avoided	To investigate the associations between hyperglycemia, cerebral metabolism and outcome	CMD-glucose levels were higher during serum glucose > 7.8 mmol/l. No differences in other microdialysis parameters were observed
(63)	Single ceter, prospective, observational	28	WFNS grade I $n = 8$ (29%), II $n = 6$ (21%), III $n = 3$ (11%), IV $n = 6$ (21%), V $n = 5$ (18%)	Monitoring was initiated 22.2 h (mean) after SAH and maintained for 195.4 h (mean)	Vascular territory of the aneurysm; insertion into lesioned tissue was avoided	To investigate the impact of hyperglycemia on cerebral metabolism	During episodes of blood glucose > 140 mg/dl, CMD-lactate and CMD-pyruvate concentrations increased, the CMD-LPR remained stable, and CMD-glutamate levels decreased. Serum glucose concentrations did not differ during episodes of high (>2.6 mmol/l) and low (<0.6 mmol/l) CMD-glucose concentrations. During episodes of low CMD-glucose, CMD-lactate, CMD-glutamate, CMD-glycerol levels, and the CMD-LPR were elevated. Low CMD-glucose during blood glucose > 140 mg/dl was associated with poor outcome

(*Continued*)

TABLE 6 | Continued

Reference	Study type	Number of patients with SAH	Patient characteristics	Monitoring period	Probe location	Study aim	Main microdialysis findings
(64)	Single ceter, prospective, observational	31	WFNS grade I *n* = 9 (29%), II *n* = 6 (19%), III *n* = 3 (10%), IV *n* = 6 (19%), V *n* = 7 (23%)	The mean duration of monitoring was 192/295 h in patients with/without insulin. Data are reported for days 1–10 after SAH	Vascular territory of the aneurysm; insertion into lesioned tissue was avoided	To investigate the impact of intravenous insulin on cerebral metabolism	CMD-glucose levels, but not serum glucose levels, decreased significantly 3 h after the start of the insulin infusion. Episodes of low CMD-glucose (<0.6 mmol/l) were (non-significantly) more common in patients who received insulin. CMD-lactate and CMD-pyruvate levels did not change, CMD-glycerol concentrations slightly increased, and CMD-glutamate levels decreased after the start of insulin treatment
(65)	Single-center, prospective, observational	24	WFNS grade I *n* = 5 (21%), II *n* = 4 (17%), III *n* = 2 (8%), IV *n* = 6 (25%), V *n* = 7 (29%)	Data were collected on days 1–10 after SAH	Vascular territory of the aneurysm; insertion into lesioned tissue was avoided	To investigate the long-term effect of insulin on cerebral metabolism	Median daily CMD-glucose levels decreased after the initiation of insulin treatment and were significantly lower, compared to baseline, 4 days after insulin start. A significant increase in CMD-glycerol levels was observed 1 day after insulin start, which was not significant thereafter. CMD-glutamate levels significantly decreased over time
(66)	Single ceter, retrospective, observational	50	Hunt and Hess grade II *n* = 3 (6%), III *n* = 7 (14%), IV *n* = 15 (30%), V *n* = 25 (50%)	Monitoring was started 2 days (median) after SAH and maintained for 108 h (mean)	Normal-appearing white matter	To elucidate the relations between enteral nutrition, insulin treatment and cerebral metabolism	There was no direct association between CMD-glucose levels and the energy content of the administered enteral nutrition. There was a significant association between CMD and serum glucose levels. When the CMD-LPR was <40, higher CMD and serum glucose levels were associated with a higher insulin dose. When the CMD-LPR was >40, a higher insulin dose was associated with lower CMD-glucose levels, despite higher serum glucose concentrations
(67)	Single ceter, prospective, observational	19	WFNS grade I *n* = 1 (5.3%), II *n* = 1 (5.3%), III *n* = 1 (5.3%), IV *n* = 11 (58%), V *n* = 5 (26%)	The mean monitoring time was 147 h. Data are reported for days 1–7 after SAH	Cortical, frontal, classified as radiologically normal-appearing or adjacent to ischemic lesions	To elucidate the relation between brain and serum glucose levels	CMD-glucose levels decreased over days 1–7. There was a significant correlation between CMD and serum glucose levels ($r = 0.27$). CMD-lactate and CMD-pyruvate levels increased over time, beginning on day 3. CMD-glutamate levels peaked on day 1 and decreased thereafter. CMD-glucose and CMD-pyruvate levels decrease during insulin treatment, despite systemic glucose concentrations within normal range

SAH, subarachnoid hemorrhage; CMD, cerebral microdialysis; LPR, lactate-to-pyruvate-ratio; GCS, Glasgow coma scale; WFNS, world federation of neurological societies.

summary, tight glycemic control may be associated with pathologically low CMD-glucose levels (neuroglucopenia) in critically ill patients with acute neurologic injury. If brain metabolic monitoring indicates critically low glucose levels (i.e., <0.2 mmol/l) targeting a more liberal glucose regimen (110–150 mg/dl or up to 180 mg/dl) may be indicated.

CMD and Outcome

Studies reporting an association of CMD parameters with patient outcome are summarized in **Table 7**. The largest study includes 182 SAH patients and found higher CMD-LPR and CMD-glutamate values during the first week after SAH being significantly and independently associated with poor functional outcome after 12 months (50). Patients with poor functional 3–6 months outcome had significantly more episodes of CMD-LPR > 40 and CMD-glutamate levels > 10 μmol/l compared to those with favorable outcome (71). An elevated CMD-LPR was also associated with hospital mortality (57). Pathologically low CMD-glucose levels < 0.7 mmol/l were observed more often in patients with poor outcome and in those who died (41, 61, 63). Elevated CMD-lactate is a less specific marker for neuroprognostication, as both, associations with good (in a hyperglycolytic context) and poor (due to hypoxia) outcome were reported (72).

Trend analysis is important as a shift to hypermetabolism, indicated by an increase in both, CMD-lactate and CMD-pyruvate levels, was observed in patients with favorable outcome. Persistent low CMD-pyruvate levels without increase to normal values were associated with poor outcome (69). Persistent low CMD-glutamate levels were associated with good functional outcome, whereas increases at day 1 and day 7 were associated with poor outcome (74).

DISCUSSION

This review demonstrates that CMD is a powerful tool providing almost continuous brain metabolic information at bedside. Based on the published literature, pathological changes in brain metabolism are associated with disease severity, mechanisms of primary and secondary brain injury, and poor long-term outcome after SAH.

As summarized in this review, the major limitation of the published literature is that CMD monitoring was reported mostly in small single-center trials of neurological and neurosurgical ICUs with extensive experience in the use of CMD as adjunct to other multimodal neuromonitoring techniques. In many centers, implementation of CMD as a clinical tool is still limited due to financial constraints and the lack of evidence to improve patient's outcome. Future trials may address this gap in the literature by targeting brain metabolic parameters as endpoints to improve brain homeostasis as no monitoring tool can improve patient's outcome unless coupled with a therapeutic intervention. On the other hand, there is a need to re-define commonly used outcome parameters and to move from a simplistic functional outcome score to a more sophisticated approach including neuropsychological testing, quality of life measures, and brain tissue outcome.

The invasiveness of CMD limits its application to poor-grade SAH patients. This implicates that this review merely summarizes brain metabolic changes of unconscious patients and results are not generalizable to all clinical grades. Based on the recently published consensus statement, the use of CMD is recommended in poor-grade SAH patients with prolonged ventilation and patients with secondary neurologic deterioration requiring mechanical ventilation.

Despite these limitations metabolic monitoring in the early and subacute phase after SAH provides insight into pathophysiological mechanisms of primary and secondary brain injury on the brain tissue level. As shown in our review, the detection of impeding ischemia is the most extensively studied application of CMD in SAH patients. CMD has been shown to have the potential to be used as early warning tool of brain tissue ischemia hours before the insult (17, 43, 44), even if clinically silent (26). In this regard, it is important to mention that we recognized a large variability in the definition of DCI throughout the published literature. Although more homogeneity was detected after the year 2010 when Vergouwen et al. defined DCI based on clinical and/or radiographic criteria (83), earlier studies are difficult to compare as information on radiographic evidence of new infarctions related to vasospasm were commonly not reported.

Another limitation is that microdialysis probe location differed in individual studies by means of targeting either brain tissue ipsilateral to the bleeding aneurysm or the contralateral hemisphere and monitoring the cortical gray matter vs. subcortical white matter. Furthermore, probe location was rarely adequately addressed as covariate in multivariate models, which limits the interpretation of results and conclusions. It is important to mention that brain chemistry can only be interpreted correctly based on the relation to focal injury on brain imaging ("normal appearing brain tissue" vs. "perilesional" vs. "intralesional") (2). As recommended by clinical experts, future trials reporting microdialysis data should at least include information on catheter location, the catheter type used, perfusion fluid composition, the perfusion fluid flow rate, and time from ictus to monitoring (2).

The interpretation of CMD-derived information requires profound knowledge of the complex underlying pathophysiology. As shown in **Table 1**, metabolic changes may be ambiguous, as similar patterns may indicate different underlying pathophysiological processes. However, taking into account, the occurrence of specific patterns in relation to the bleeding onset may help to discriminate between early and late onset ischemic patterns or patterns of mitochondrial dysfunction. A clinical guidance is given in **Table 1** and may be combined with other neuromonitoring parameters such as brain tissue oxygenation, ICP, and CBF.

Clinical implication of microdialysis monitoring besides the detection of secondary ischemic insults include guidance of systemic glucose management, CPP optimization, defining individual transfusion thresholds and monitoring of brain chemistry during pharmacological and non-pharmacological interventions (41, 49, 51, 52, 57, 61, 63, 67).

The only treatment decision based on changes in brain metabolism currently recommended by clinical experts is the treatment of low cerebral glucose taking into account baseline

TABLE 7 | Brain metabolism and outcome after SAH.

Reference	Study type	Number of patients with SAH	Patient characteristics	Monitoring period	Probe location	Study aim	Main microdialysis findings
(68)	Single-center, prospective, observational	28	Hunt and Hess grade II $n = 3$ (11%), III $n = 6$ (21%), IV $n = 3$ (11%), V $n = 16$ (57%)	Monitoring was initiated 1 day (median) after SAH. Data are reported up to 12 days after SAH	White matter at greatest risk for secondary brain injury; classified as normal or perilesional tissue	Investigating associations between CMD-K^+ levels, brain metabolism and functional outcome	Elevated cerebral CMD-K^+ levels (above the median of 3 mmol/l) were associated with CMD-LPR > 40, CMD-lactate > 4 mmol/l and poor outcome. CMD-K^+ concentrations positively correlated with CMD-lactate and CMD-glutamate levels and the CMD-LPR. CMD-LPR > 40 was independently associated with poor functional outcome
(69)	Single-center, prospective, observational	20	Hunt and Hess grade III $n = 10$, IV $n = 6$, V $n = 4$	CMD monitoring started within 24 h after SAH in most patients and was maintained for 3–12 days (range)	Cortical, frontal	Describing metabolic profiles in relation to functional outcome	A metabolic pattern of decreasing CMD-glucose levels paralleled by an increase in both, CMD-lactate and CMD-pyruvate concentrations, after 24–72 h was common in patients with good outcome. A pattern of CMD-glucose levels remaining high combined with low CMD-pyruvate concentrations was common in patients with poor outcome
(70)	Single-center, prospective, randomized-controlled	30/60	GCS score average of 5	n/a	n/a	Comparing an ICP-based to a CPP-based treatment concept	Patients with poor outcome had significantly lower levels of CMD-glucose (1.1 vs. 2.1 mmol/l) and higher levels of CMD-glycerol and a higher CMD-LPR compared to patients with good outcome
(14)	Single-center, prospective, observational	26	Hunt and Hess grade II $n = 2$ (7.7%), III $n = 6$ (23.1%), IV $n = 2$ (7.7%), V $n = 16$ (61.5%)	Monitoring was started 22 h (median) after SAH and data of the following 144 h are reported	Frontal, ipsilateral to the aneurysm; classified as normal-appearing or perilesional brain tissue	Describing the metabolic profile during the early phase after SAH	A higher CMD-LPR was associated with poor outcome
(15)	Single-center, prospective, observational	39	Hunt and Hess grade I + II $n = 3$ (8%), III $n = 6$ (15%), IV $n = 12$ (31%), V $n = 18$ (46%)	Data are reported for days 2–10 after SAH	Frontal, contralateral to the craniotomy in clipped patients; non-dominant hemisphere in diffuse SAH or ipsilateral in lateralized SAH in coiled patients	Comparing brain metabolism of patients with and without global cerebral edema on admission	CMD-LPR > 40 and metabolic crisis (CMD-LPR > 40 and CMD-glucose < 0.7 mmol/l) were associated with poor outcome
(57)	Single ceter, prospective, observational	28	Hunt and Hess grade III $n = 6$ (21%), IV $n = 8$ (29%), V $n = 14$ (50%)	Monitoring was initiated on day 2 (median) after SAH and maintained for 6 days (median)	White matter, frontal, tissue at risk or contralateral to the craniotomy	To assess the relationship between rapid reductions in serum glucose and brain metabolism	A higher CMD-LPR was associated with hospital mortality
(71)	Single-center, prospective, observational	35/40	Admission WFNS grade IV + V $n = 22$	Data are reported for days 2–17 after SAH. The mean monitoring time per patient was 4 days	Frontal or suspected aneurysmal vascular territory	Investigating the association between brain metabolism and patient outcome	Patients with unfavorable outcome showed a higher CMD-LPR compared to those with good outcome. Episodes of CMD-LPR > 40 or CMD-glutamate levels > 10 μmol/l were both associated with cerebral infarction and poor outcome

(*Continued*)

TABLE 7 | Continued

Reference	Study type	Number of patients with SAH	Patient characteristics	Monitoring period	Probe location	Study aim	Main microdialysis findings
(50)	Single-center, prospective, observational	182	WFNS grade 0 *n* = 3 (2%), I *n* = 61 (37%), II *n* = 23 (14%), III *n* = 17 (10%), IV *n* = 35 (21%), V *n* = 25 (15%)	Monitoring was started 23/13 h (mean) after SAH and maintained for 169/172 h (mean) in patients with/without intracranial hypertension	Vascular territory of the aneurysm; insertion into lesioned tissue was avoided	Investigating the impact of intracranial hypertension on cerebral metabolism	Higher CMD-LPR and CMD-glutamate levels were associated with poor outcome
(61)	Single ceter, prospective, observational	10/20	GCS score 7 (median), 3–10 (range)	CMD monitoring was started 45 h (median) after SAH and maintained for 96 h (median)	Frontal, near the area of lesioned tissue or right frontal lobe in patients with diffuse injury	Investigating the impact of tight glycemic control on cerebral metabolism	Blood glucose levels were defined as "tight" (4.4–6.7 mmol/l) or "intermediate" (6.8–10 mmol/l). Tight blood glucose was associated with lower levels of CMD-glucose, more episodes of CMD-glucose < 0.7 mmol/l, higher CMD-LPR and a more frequent occurrence of metabolic crisis (CMD-LPR > 40 and CMD-glucose levels < 0.7 mmol/l). Low CMD-glucose levels and metabolic crisis were associated with higher hospital mortality
(51)	Single-center, prospective, observational	18	Hunt and Hess grade II *n* = 2, III *n* = 4, IV *n* = 8, V *n* = 4	Monitoring was initiated 1 day (median) after SAH. Between 37 and 168 hourly samples were obtained per patient.	White matter, contralateral to the maximal focal injury, normal-appearing tissue	To assess the impact of induced normothermia on cerebral metabolism	When normothermia (37°C) was induced due to refractory fever (≥38.3°C), it was associated with a decrease in CMD-LPR and fewer episodes of metabolic distress (CMD-LPR > 40). Patients with poor outcome had a higher CMD-LPR
(72)	Two-center, prospective, observational	31	Comatose patients	Monitoring was started 1 day (median) after SAH and maintained for 7 days (median)	Visually normal white matter	To elucidate the relevance of elevated CMD-lactate levels in the context of brain tissue hypoxia and hyperglycolysis	Elevated CMD-lactate concentrations (>4 mmol/l) were defined as "hypoxic" (together with $P_{bt}O_2$ < 20 mmHg), "hyperglycolytic" (together with CMD-pyruvate concentrations > 119 μmol/l), or neither. Hyperglycolytic elevated CMD-lactate was more common than hypoxic elevated CMD-lactate and associated with favorable outcome. Hypoxic elevated CMD-lactate was associated with an increased mortality
(73) (abstract only)	Single-center, prospective, observational	18/51	n/a	n/a	n/a	To correlate CMD findings with functional outcome	Poor 12-month outcome was correlated with lower CMD-glucose levels and higher levels of CMD-glycerol and CMD-LPR
(33)	Single-center, prospective observational	10	Hunt and Hess grade II *n* = 3 (30%), III *n* = 4 (40%), IV *n* = 3 (30%)	Start time is not exactly given (figures indicate 12–30 h after SAH). Monitoring lasted 6–11 days (range)	Cortical, frontal	To match CMD data with CT findings, clinical course and outcome	CMD-lactate elevations were frequently observed without obvious cause, while CMD-LPR reflected ischemia and, during days 0–4, correlated with outcome. In an infracted area, CMD-glucose levels fell to and remained at 0. Zero-levels of CMD-glucose were observed more frequently in patients with poor outcome. Patients with poor outcome had significantly higher CMD-glutamate levels

(*Continued*)

TABLE 7 | Continued

Reference	Study type	Number of patients with SAH	Patient characteristics	Monitoring period	Probe location	Study aim	Main microdialysis findings
(18)	Single-center, prospective, observational	149	WFNS grade 0 *n* = 3 (2%), I *n* = 53 (36%), II *n* = 16 (11%), III *n* = 17 (11%), IV *n* = 33 (22%), V *n* = 27 (18%)	Monitoring was started after aneurysm treatment (mean 24.7 h after SAH) and maintained for 161.8 h (mean)	Vascular territory of the aneurysm; insertion into lesioned tissue was avoided	Investigating the relationship between clinical disease severity, brain metabolism and outcome	Higher CMD-glutamate levels and CMD-LPR were independently associated with poor functional outcome and mortality
(20)	Single-center, prospective, observational	26	Hunt and Hess grade II *n* = 1 (4%), III *n* = 7 (26%), IV *n* = 2 (8%), V *n* = 16 (62%)	Monitoring was started 1 day (median) after SAH and maintained for 4 days (median)	Vascular territory of the aneurysm; classified as normal-appearing or perilesional brain tissue	To investigate the association between neuroinflammation, axonal injury and brain metabolism	High-grade neuroinflammation (CMD-IL-6 levels above median) was associated with poor functional outcome
(63)	Single ceter, prospective, observational	28	WFNS grade I *n* = 8 (29%), II *n* = 6 (21%), III *n* = 3 (11%), IV *n* = 6 (21%), V *n* = 5 (18%)	Monitoring was initiated 22.2 h (mean) after SAH and maintained for 195.4 h (mean)	Vascular territory of the aneurysm; insertion into lesioned tissue was avoided	To investigate the impact of hyperglycemia on cerebral metabolism	Low CMD-glucose levels during episodes of blood glucose concentrations > 140 mg/dl was associated with poor outcome
(41)	Single-center, prospective, observational	30	Hunt and Hess grade II + III *n* = 5 (17%), IV *n* = 10 (33%), V *n* = 15 (50%)	Monitoring was started 3 days (median) after SAH and maintained for 110 h (median)	Frontal, ipsilateral to lateralized aneurysms, right frontal lobe in case of midline aneurysms	To identify the relation between CPP thresholds and brain metabolic crisis	Metabolic crisis (CMD-LPR > 40 and CMD-glucose concentrations ≤0.7 mmol/l) was associated with poor outcome
(74)	Single-center, prospective, observational	10	Hunt and Hess grade I *n* = 2, II *n* = 3, III *n* = 1, IV *n* = 2, V *n* = 2	CMD was performed for 1.7–7 days (range). Data are reported up to 9 days after SAH	Frontal	Investigating associations between cerebral metabolism and patient outcome	Higher concentrations of CMD-glutamate and CMD-lactate were associated with poor functional outcome. In patients with poor outcome, glutamate levels followed a biphasic course with peaks on days 1–2 and 7. In patients with good outcome, glutamate levels remained low without any temporal dynamic
(75)	Single-center, prospective, observational	18	WFNS grade IV *n* = 4 (22%), V *n* = 14 (78%)	The median monitoring time was 8 days	Cortical, vascular territory of the aneurysm	Comparing CMD values to the arterio-jugular difference	No significant differences in absolute values of CMD parameters were observed between patients with good and poor outcome. CMD-lactate levels >4 mmol/l and CMD-pyruvate levels >119 μmol/l were significantly more common in patients with good outcome. Hypoxic elevated lactate ($P_{bt}O_2$ < 20 mmHg) was more common in patients with poor outcome

SAH, subarachnoid hemorrhage; CMD, cerebral microdialysis; LPR, lactate-to-pyruvate-ratio; GCS, Glasgow coma scale; n/a, data not available; ICP, intracranial pressure; CPP, cerebral perfusion pressure; $P_{bt}O_2$, brain tissue oxygen tension; CBF, cerebral blood flow; IL-6, interleukin-6.

TABLE 8 | CMD findings not directly related to the discussed topics.

Reference	Study type	Number of patients with SAH	Patient characteristics	Monitoring period	Probe location	Study aim	Main microdialysis findings
(76) (abstract only)	n/a	n/a	n/a	Data are reported between 2 and 12 days after SAH	n/a	Investigating the effect of remote ischemic preconditioning on brain metabolism	Over the duration of remote ischemic preconditioning, the CMD-LPR and CMD-glycerol levels decreased. The effect persisted for 25–54 h
(77)	Single-center, prospective, observational	5	WFNS grade I *n* = 5 (100%)	All patients were operated within 1–3 days after SAH	Frontal or temporal, vascular territory most likely affected by vasospasm	Describing CMD levels in good-grade SAH patients	Mean levels of CMD parameters in WFNS grade I patients were: CMD-glucose 2.6 mmol/l, CMD-lactate 2.9 mmol/l, CMD-pyruvate 92 µmol/l, CMD-LPR 36, CMD-glutamate 11.2 µmol/l
(78)	Single-center, prospective, observational	17	WFNS grade I *n* = 1, II *n* = 3, III *n* = 5, IV *n* = 3, V *n* = 5	Probes were inserted within 72 h after SAH. Monitoring was performed for 85–336 h (range)	Vascular territory of the aneurysm; insertion into lesioned tissue was avoided	Investigating the association between CSD and brain metabolism	Patients with acute focal neurological deficits had higher baseline CMD-glutamate and CMD-lactate levels compared to those without. In patients without acute focal deficits, there was a significant transient decrease in CMD-glucose (−25%) and increase in CMD-lactate concentrations (+10%) following clusters of CSDs (2 or more per hour), but not single CSDs. No changes in CMD-glutamate levels were observed
(79) (abstract only)	Bi-center, prospective, observational	21	WFNS grade I–III *n* = 11, IV–V *n* = 10	Data are reported up to 14 days after SAH	Vascular territory at risk for DCI	To investigate an association between CSD and CMD-glucose level	CSD were not associated with changes in CMD-glucose levels
(80)	Single-center, retrospective, observational	167	WFNS grade 0 *n* = 3 (2%), I *n* = 58 (35%), II *n* = 21 (13%), III *n* = 18 (11%), IV *n* = 38 (23%), V *n* = 29 (17%)	Data are reported for days 1–10 after SAH. CMD was performed for 7–10 days	Vascular territory of the aneurysm; patients were excluded if the tip of the CMD probe was close to a parenchymal hemorrhage	To relate changes in cerebral metabolism to the emergence of bacterial meningitis	On the day when bacterial meningitis was diagnosed, CMD-glucose levels and the CMD-lactate-to-glucose-ratio were significantly lower than 3 days before. Compared to controls, only the decrease in CMD-glucose levels was more pronounced in patients with bacterial meningitis. A decrease in CMD-glucose levels of 1 mmol/l showed the highest combined sensitivity and specificity
(81) (Abstract only)	Single-center, prospective, observational	4	n/a	n/a	n/a	Investigating the impact of temperature changes on CMD-glutamate levels	In all patients, mild head cooling resulted in a significant decrease in CMD-glutamate levels. In 2 patients, CMD-glutamate concentrations increased sharply with fever
(82)	Single-center, prospective, observational	6/18	GCS motor score 3 *n* = 3, 5 *n* = 1, 6 *n* = 2	n/a	Cortical, normal-appearing tissue	To assess the impact of fever on cerebral metabolism	Neither the onset of fever (≥38.7°C) nor its resolution was associated with significant changes in cerebral metabolism

n/a, data not available; SAH, subarachnoid hemorrhage; CMD, cerebral microdialysis; LPR, lactate-to-pyruvate-ratio; WFNS, world federation of neurological societies; CSD, cortical spreading depolarizations; DCI, delayed cerebral ischemia.

systemic glucose concentration, catheter location, and the etiology of neuroglucopenia. In the knowledge of its potential, there is a need to integrate brain metabolic changes and to define CMD-based endpoints in future clinical trials.

CONCLUSION

Cerebral microdialysis is used in the clinical management of severe SAH together with ICP, $P_{bt}O_2$, and other neuromonitoring parameters. In the knowledge of its limitations, this method provides a novel insight into pathophysiological processes of primary and secondary brain injury. Recent consensus on microdialysis monitoring paves the way for improved protocols and targeted interventions. The major task for future research integrates the prospective evaluation of predefined interventions to improve brain tissue physiology aiming toward a personalized management of SAH patients in the future.

AUTHOR CONTRIBUTIONS

RH was involved in the idea, design, data acquisition, article selection, writing, interpretation of data, and final revision of the manuscript. MK was involved in article selection, writing, interpretation of data, and final revision of the manuscript. AS, MG, and VR were involved in the data acquisition, interpretation of data, and final revision of the manuscript. BP, RB, and ES were involved in article selection, writing, interpretation of data, and final revision of the manuscript. All authors read and approved the final version of the manuscript and are accountable for the content, data interpretation, and data accuracy.

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REVIEW
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Current and Emerging Technologies for Probing Molecular Signatures of Traumatic Brain Injury

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Traumatic brain injury (TBI) is understood as an interplay between the initial injury, subsequent secondary injuries, and a complex host response all of which are highly heterogeneous. An understanding of the underlying biology suggests a number of windows where mechanistically inspired interventions could be targeted. Unfortunately, biologically plausible therapies have to-date failed to translate into clinical practice. While a number of stereotypical pathways are now understood to be involved, current clinical characterization is too crude for it to be possible to characterize the biological phenotype in a truly mechanistically meaningful way. In this review, we examine current and emerging technologies for fuller biochemical characterization by the simultaneous measurement of multiple, diverse biomarkers. We describe how clinically available techniques such as cerebral microdialysis can be leveraged to give mechanistic insights into TBI pathobiology and how multiplex proteomic and metabolomic techniques can give a more complete description of the underlying biology. We also describe spatially resolved label-free multiplex techniques capable of probing structural differences in chemical signatures. Finally, we touch on the bioinformatics challenges that result from the acquisition of such large amounts of chemical data in the search for a more mechanistically complete description of the TBI phenotype.

Keywords: traumatic brain injury, microdialysis, proteomics, lipidomics, metabolomics, Raman spectroscopy

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INTRODUCTION

In broad terms, we understand the biology of traumatic brain injury (TBI) as being the result of an interplay between the biomechanics of the initial insult and resulting injury, the effects of the subsequent resuscitative treatments and the host response. These latter factors are important; even the best available prognostic models predict only a small part of the observed variability in outcome (1). The host response is at least in part genetically determined and a number of stereotypical molecular mechanisms are implicated in the pathobiology of TBI (2). Nevertheless, the patient population is highly heterogeneous and our understanding of the biochemistry of how traumatic lesions go on to influence neural connectivity and brain function is very incomplete. An important related issue is the translational gap: time and again successful experimental interventions in animal models have failed

to translate into clinical therapies. Even accepted resuscitative and intensive care strategies are blunt instruments with a wide range of harmful side effects (2). A more nuanced understanding of pathobiology is crucial if novel therapeutics and individualized treatment strategies are to be realized.

One possible explanation for the translational failures to date is that our understanding of the detailed pathological mechanisms is incomplete or that our clinical phenotype is insufficiently refined to meaningfully characterize the biological state of the patient in a therapeutically meaningful way. Parallel exploration of mechanisms in the clinical setting through direct longitudinal focal monitoring and neuroimaging, and in the laboratory is essential to understand the heterogeneity of TBI, identify biomarkers of injury evolution/restoration, refine experimental models, and develop neurorestorative treatments. A large number of techniques are now available for assessing molecular patterns of TBI in both the experimental and clinical setting (**Figure 1**). Current technologies, including microdialysis (MD) and various chemically sensitive imaging techniques [such as magnetic resonance (MR) spectroscopy and positron emission tomography] may overlap with emerging technologies such as proteomic and metabolomic approaches or optical spectroscopy.

The molecular signatures generated by emerging experimental techniques are complex and new approaches will likely be needed to make sense of the large amount of data that are becoming available. However, in the post-genomic era, there has been an explosion in technologies exploiting informatics approaches to make the interpretation of biological "big data" tractable. These have had a huge impact on the discovery of both next-generation diagnostics and also new biomarkers for precision medicine approaches to research and treatment. The fundamental premise is that the evolutionary complexity of biological systems renders them difficult to comprehensively understand using only a reductionist approach. Such complexity can become tractable with the use of '*omics* research; this term implies the study of entities in aggregate. Similar considerations apply to making sense of the large volumes of experimental data provided by novel biochemically sensitive techniques.

We will review the kinds of biochemical data that can now be accessed experimentally and explore possible future trends that will allow a better understanding of mechanisms and timing of disease progression and pave the way for personalized medicine in the treatment of TBI.

MULTIPLE-ANALYTE APPLICATIONS OF CEREBRAL MD

Technical Aspects

Microdialysis allows continuous sampling of endogenous substances in the interstitial fluid of a target organ (e.g., the brain). The principles of MD have been extensively reviewed

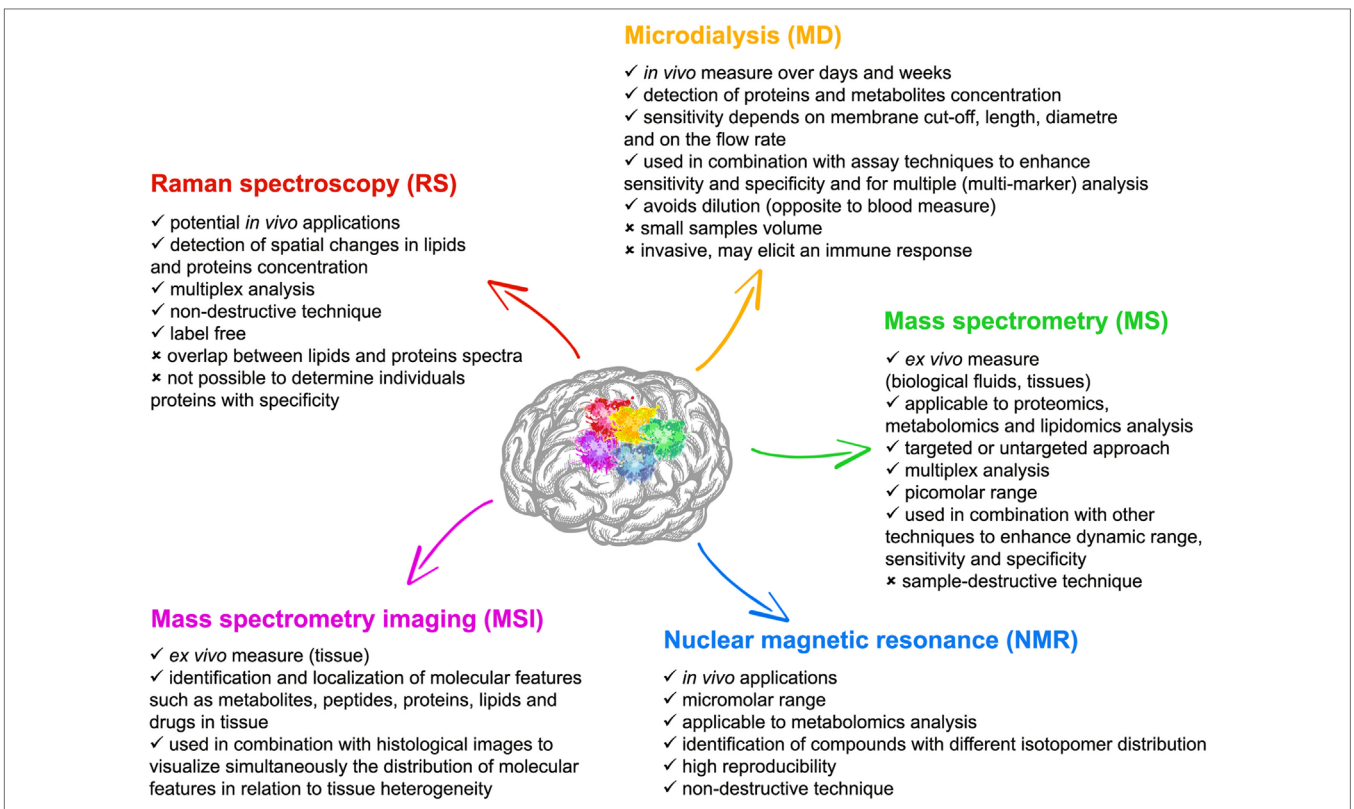

FIGURE 1 | Features of the five main experimental techniques with multiplex chemical sensing capability described in this review; microdialysis (MD), mass spectrometry (MS) [and mass spectrometry imaging (MSI)], nuclear magnetic resonance (NMR), and Raman spectroscopy (RS).

elsewhere [for example, Ref. (3)]. Briefly, perfusion fluid is pumped through the inner tube of the MD probe, which has a concentric design and is semipermeable. At the tip of the probe, substances in the interstitial fluid of the target tissue can diffuse along the concentration gradient to some extent equilibrating with the perfusion fluid (4). The resulting dialysate is collected externally in vials.

Compared with the other technologies for assessing molecular patterns of TBI described in this review, MD offers several practical advantages. Most significantly, compounds can be measured semi-continuously *in vivo* over several hours, days, or even for weeks. In addition, MD captures the molecules in the interstitial space at the site of their cellular actions and is, therefore, not subject to dilution such as occurs when sampling other fluids such as blood or cerebrospinal fluid (CSF). MD technology still has its limitations, including its focal resolution since MD samples very small volumes of tissue and its invasiveness [although the effects of catheter microtrauma are transient and reversible and likely of little effect compared to pathological processes (5)]. Moreover, MD may elicit an immune response resulting in altered microcirculation, blood–brain barrier (BBB) damage and perturbation of brain metabolism (3, 6). Another potential limitation of MD is relative substance recovery, an inherent aspect of MD sampling, which implies that substance concentration in the MD samples represent only a fraction of the total amount in the extracellular space. Recovery depends largely on perfusion flow rate (the lower the flow rate, the higher the recovery) and the length and diameter of the membrane. Other factors related to recovery are the diffusion coefficients of the various substances and their interaction with the dialysis membrane (3). At the typically low flow rates used in human MD studies (0.3 μL/min), the sampling time is sufficient for complete equilibration between the perfusion fluid and the surrounding environment to occur: recovery of small molecular weight substances, such as neurotransmitters and energetic compounds (i.e., glucose, lactate, and pyruvate) is very good. However, larger molecules, such as cytokines, chemokines, and other CNS proteins (e.g., axonal proteins), tend to have lower relative recoveries (7, 8). Sensitive analytical methods (i.e., immunoassays) are required to counteract such poor analyte recovery and small sample volumes, another potential limitation of MD. Nonetheless, protein sampling is challenging, and methods to optimize protein recovery with high cut-off membranes are under investigation. Serum albumin, which is widely used in the perfusion fluid, may be used to reduce non-specific binding of the proteins of interests to tubing and membranes, allowing consistent protein recovery (7). Another approach to improve MD recovery is to coat the membranes with substances (i.e., pluronic), again aimed at reducing protein binding (9, 10).

Over time, the MD catheter surface becomes coated with a biofilm due to an inevitable stereotyped inflammatory response to indwelling implants, and this has been demonstrated after 8 days by electron microscopy (11). As a result, a drop-off in recovery *in vivo* may be expected (12). However, such a reduction in performance over time has not been consistently demonstrated (11) and clinical use over prolonged periods is commonplace. The measurement of a small diffusible molecule such as urea whose plasma concentration can be conveniently tracked and is related to brain interstitial concentration has been suggested as an indicator of MD performance (13).

While MD is relatively safe, it is a highly invasive technique and is, therefore, intrinsically unsuitable for use in mild TBI. For similar reasons, it is difficult to obtain normative data from truly "normal" brain that limits our physiological understanding although MD has been applied to patients who have undergone elective neurosurgery in an attempt to obtain better reference ranges (14).

Clinical and Translational (Research) Applications of MD

Microdialysis was originally developed to measure cerebral neurotransmitters (15). Over the last decade, cerebral MD has been increasingly used in the clinical setting as an established technique for continuous monitoring of substrate delivery and metabolism in patients with TBI and other acute brain injuries. In addition, MD continues to be a powerful translational research tool to explore cerebral physiological and pathophysiological mechanisms. This topic will be the main focus of this section and cited studies are summarized in **Table 1**.

The clinical use of MD to monitor brain chemistry in neurointensive care has the topic of excellent reviews (24, 25). Clinical bedside MD analyzers using colorimetric enzymatic assays are commercially available for measurement of compounds related to brain energy metabolism, including glucose, lactate, and pyruvate as well as glycerol and the amino acid glutamate, although these are less commonly used clinically. The lactate/pyruvate ratio (LPR) is commonly calculated as an indicator of failing aerobic metabolism and, additionally, has the advantage of being insensitive to recovery fraction since both numerator and denominator are affected approximately equally.

Traumatic brain injury results in well defined metabolic disturbances (i.e., metabolic crisis) characterized by high LPR, high glutamate, and low glucose levels. These metabolic patterns may occur despite adequate resuscitation and controlled intracranial pressure (ICP) (26) and may also be associated with nonconvulsive seizures, periodic discharges, and cortical spreading depolarization (27, 28). Metabolic perturbation is related to clinical outcome and is affected by interventions (29–34). Such metabolic traits are of interest since the restoration of metabolic homeostasis is a key goal of intensive care. Elevations in LPR are increasingly clinically accepted as a metabolic marker with a potential to direct therapeutic interventions (25). However, LPR cannot be univocally interpreted as a sign of ischemia. Elevated LPR in the absence of ischemia with normal or high pyruvate concentration has been also described. This may reflect mitochondrial dysfunction (35). LPR elevation with low oxygenation characterizes post-TBI ischemia, whereas metabolic crisis as LPR elevation but with normal or high oxygenation is associated with profound mitochondrial dysfunction but a normal supply of energetic substrate. These two aspects need to be distinguished since augmenting oxygen and substrate delivery in the context of mitochondrial failure will be at best ineffective and could potentially be harmful.

TABLE 1 | Research applications of MD.

Reference	Patients characteristics	Time (after injury)	Biosample	Techniques employed	Key findings
Magnoni et al. (8)	TBI, GCS <8 (16 TBI, no controls)	12–96 h	ECF (contusional and normal appearing brain)	100 kDa MD + serial ELISA (no sample pooling)	Elevated tau in brain ECF correlates with reduced amyloid-β levels and predicts adverse clinical outcome. Tau and NFL levels were 4-fold higher in patients with MD catheters placed in pericontusional regions than those with MD in normal-appearing frontal lobe
Magnoni et al. (5)	TBI, GCS <8, no controls	13–96 h	ECF (contusional and normal appearing brain)	100 kDa MD + serial ELISA (no sample pooling)	Acute tau in brain ECF correlated with DTI measurements of reduced brain white matter integrity in white matter-masked region near the MD catheter
Helmy et al. (7)	TBI, GCS <8 (12 TBI, no controls)	<96 h–5 d	ECF, plasma	100 kDa MD + Multiplex ELISA (sample pooling)	Cytokine production is highly compartmentalized, with quantitative and qualitative differences between brain parenchymal and systemic cytokine concentrations
Helmy et al. (16)	TBI, GCS <8 (20 TBI, no controls)	24 h–5 d	ECF (normal appearing brain), plasma	reverse 100 kDa MD + Multiplex ELISA (sample pooling)	Subcutaneously administered rhIL-1Ra results in a large increase in concentration of this cytokine both in the circulation and in the brain ECF, in TBI patients. rhIL-1Ra treatment modulates the brain extracellular cytokine and chemokine profile
Marklund et al. (17)	TBI, GCS <8 (8 TBI, no controls)	1–5 d	ECF	100 kDa MD + standard ELISA (sample pooling)	Patients with a predominantly focal lesion show higher ECF tau than those with DAI, 1–3 d post injury. Patients with DAI show consistently higher amyloid-β42 levels than those with focal injury
Guilfoyle et al. (18)	TBI, GCS < 8 (12 TBI, controls: paired catheteters in normal appearing brain)	24–48 h to 5 d	ECF (contusional and normal appearing brain)	100 kDa MD + Multiplex ELISA (sample pooling)	Early and localized increase in MMP-9 concentration within pericontusional brain post-TBI is indicative of BBB damage and edema formation
Petzold et al. (19)	TBI, GCS median 9 (10 TBI, no controls)	3 h–5 d	ECF (contusional and normal appearing brain)	100 kDa MD + standard ELISA and Gel electrophoresis of (sample pooling)	Quantification of specific protein biomarkers (NfH476-986 and NfH476-1026) applicable to *in vivo* monitoring of diffuse axonal injury and neuronal loss in TBI
Lakshmanan et al. (20)	TBI, GCS <8, or a GCS of 9–14 with contusion on CT scan. (2 TBI with normal LPR and 3 with LPR >40.)	<4 d	ECF(contusional and normal appearing brain), serum	20 kDa MD + peptidomics and proteomics approaches based on different MS platforms (MALDI–TOF MS, LC-MS/MS)	Quantification of protein fragments in the ECF. Metabolic distress after TBI is associated with a differential proteome that indicates cellular destruction during the acute phase. This suggests that metabolic stress has immediate cellular consequences after TBI
Jalloh et al. (21)	TBI, GCS <8 (9 TBI)	1–7 d	ECF (normal appearing)	Reverse 100 kDa MD with ^{13}C-labeled compounds + *ex vivo* NMR of ^{13}C-labeled metabolites	Lower lactate/pyruvate ratio suggests better redox status: cytosolic NADH recycled to NAD^+ by mitochondrial shuttles. Direct tricarboxylic acid cycle supplementation with 2,3-$^{13}C_2$ succinate improved TBI brain chemistry, indicated by biomarkers and ^{13}C-labeling patterns in metabolites
Orešič et al. (22)	TBI, GCS <8 (5 TBI, no controls)	Acutely after TBI	ECF, serum	100 kDa MD + MS-based metabolomics (GC × GC-TOF-MS)	TBI is associated with a specific metabolic profile in serum which is also reflected in brain ECF (MD samples), which is exacerbated proportionally to the severity of TBI. Top ranking serum metabolites associated with TBI were found highly correlated with their MD levels suggesting possible sensitivity to BBB damage, as well as protective response and altered metabolism post-TBI
Reference	**Injury model characteristics**	**Time (after injury)**	**Biosample**	**Techniques employed**	**Key findings**
Dahlin et al. (23)	Model of progressive ICP increase leading to brain death (swine)	<12 h	ECF	100 kDa MD + proteomics by iTRAQ and nanoflow LC-MS/MS (sample pooling)	Definition of *in vivo* performance of a refined MD method, including catheter surface modification, for protein biomarker sampling in a clinically relevant porcine brain injury model. Surface modified MD show improved extraction efficiency for most of the proteins compared to naïve MD catheters

Patient characteristics describes the injury severity level according to GCS, and the number of patients/controls included in the study. Injury model characteristics describes the experimental TBI model used. Time after injury refers to the sampling time point/s or window. Biosample specifies the sample used for the analysis. Techniques employed describes the technique and/or assay used for the analysis. Key findings highlight any specific insights or notable findings in the papers.

BBB, blood–brain barrier; CT, computed tomography; d, day/s; DAI, diffuse axonal injury; DTI, diffusion tensor imaging; ECF, extracellular fluid; ELISA, enzyme-linked immunosorbent assay; GC, gas chromatography; GCS, Glasgow coma scale; h, hour/s; ICP, intracranial pressure; iTRAQ, isobaric tags for relative and absolute quantification; LC, liquid chromatography; LPR, lactate pyruvate ratio; MALDI, matrix-assisted laser desorption/ionization; MD, microdialysis; MMP-9, matrix metallopeptidase 9; MS, mass spectrometry; NAD^+, nicotinamide adenine dinucleotide (oxidized form); NADH, Nicotinamide adenine dinucleotide (reduced form); NfH, neurofilament heavy chain; NFL, neurofilament light chain; NMR, nuclear magnetic resonance; rhIL-1Ra, recombinant human interleukin-1 receptor antagonist; TBI, traumatic brain injury; TOF, time of flight.

Microdialysis is also currently the only technique for measuring the concentration of putative neuroprotective drugs directly in the brain parenchyma of patients in clinical pharmacological studies (36). Helmy et al. used this technique to demonstrate cerebral penetration of an interleukin-1 receptor antagonist and explore its biological effects by downstream MD sampling of cytokines and chemokines (16). Similarly, Mazzeo et al. used MD to assess the effect of cyclosporin on brain energy metabolism in a randomized, double blind, placebo-controlled study on 50 patients with severe brain injury (37).

Novel, elegant studies have combined ^{13}C-labeled MD and detection with *ex vivo* nuclear magnetic resonance (NMR) or *in vivo* MR spectroscopy to provide insights into important biochemical pathways in the human brain (38). Notably, these studies provided evidence that lactate can be used as substrate *in vivo* and that direct tricarboxylic acid cycle supplementation with 2,3-$^{13}C_2$ succinate improves brain chemistry after TBI (21). Both the pharmacological and labeling studies are examples of novel translational medicine approaches to the mechanistic investigation of putative therapeutic strategies and neuroprotective drugs. Along similar lines, pilot interventional studies have used MD to demonstrate beneficial effects of exogenous lactate supplementation in patients with TBI (30).

Microdialysis may also be used to measure *in vivo* changes in protein biomarker concentrations after TBI (9, 25, 39). Such studies require catheters with a higher permeability cutoff. Colloids such as dextran or albumin solutions are usually added to the standard perfusion fluid to increase both fluid and protein recovery (7, 10). The small volumes collected by standard hourly sampling (at 0.3 μL/min flow rate) are rarely sufficient to measure biomarkers of interest using standard ELISA techniques. Samples may be pooled, but this reduces time resolution (17). Alternatively, high sensitivity ELISA, multiplex approaches (i.e., Luminex technology) and sequential ELISA methods (where, instead of discarding the solution after incubation, a portion is sequentially transferred to further ELISA plates with different antibodies so that unbound analytes can be captured) have been applied in an attempt to overcome this limitation and measure several biomarkers simultaneously with the traditional hourly or two-hourly sampling frequency (7, 8, 18). There is a limit to the number of biomarkers that can be simultaneously measured from the same sample. However, proximity ligation assay (PLA) and proximity extension assay (PEA) technologies are currently being investigated for the simultaneous analysis of more than 100 biomarkers in MD samples (9). PLA technology has superior sensitivity compared to conventional sandwich assays, and it is likely that this methodology will confer a significantly better time resolution.

Recent MD studies have showed that the brain extracellular fluid contains increased concentrations of tau, neurofilament light chain (NF-L), and neurofilament heavy chain (NF-H), all of which are normally cytosolic axonal proteins (8, 17, 19) but which may be released into the extracellular space after TBI as result of axonal injury. Magnoni et al. placed MD catheters in 16 patients with severe TBI and demonstrated the feasibility of quantitative assessment of the axonal proteins NF-L and tau with a 1–2 h time resolution. Importantly, they also found a positive correlation between initial tau levels and poor clinical outcome, likely reflecting the degree of axonal injury (8). Comparable results have also been obtained from measuring tau with standard ELISA, albeit with reduced time sensitivity (17). Similarly, Petzold et al. used high cut-off MD to measure the levels of extracellular fluid NF-H fragments and monitor diffuse axonal injury and neuronal loss *in vivo* after TBI (19). These MD-based assessments of biomarkers of TBI require further validation but could be useful for early phase clinical pharmacodynamic testing of candidate therapeutics targeting axonal injury after TBI. Current technology is not suitable for bedside application. However, improvements to the speed and sensitivity of protein biomarker measurements may change this landscape in the near future. Future studies using methods such as rapid biomarker sampling combined with enhanced analytical techniques and/or novel pharmacological tools could provide additional information on the importance of tau and other proteins in both the acute pathophysiology and long-term consequences of TBI.

Helmy et al. used MD to characterize the cytokine signatures after severe TBI in 12 patients over 5 days using an analytical multiplex approach (i.e., Luminex technology) (7, 40). Forty-two cytokines were measured. Notably, the extracellular fluid concentrations of several of the explored cytokines were significantly higher than plasma concentrations and showed a stereotyped temporal peak, indicating local production.

Several matrix metalloproteinases (MMPs) have also been measured in MD samples from paired MD catheters in pericontusional vs radiologically "normal" sites using a multi-analyte profiling kit. For proteins detection, 8-h samples were pooled together. This study showed that MMP-9 concentrations are increased in pericontusional brain early post-TBI, likely reflecting post- traumatic proteolytic breakdown of the BBB which correlates with hemorrhagic progression and vasogenic edema (18).

At present, clinical MD bedside analyzers employ simple colorimetric assays for the quantification of energetic-related molecules. Bedside MD assessment of protein biomarkers with similarly convenient automated immunoassays would be very attractive for TBI research and translation into clinical practice, when available (41).

MD Combined with Proteomics and Metabolomics

One new application of MD is in the comparison of protein and metabolic profiles of extracellular fluids in healthy and diseased subjects using proteomic and metabolomic approaches. Compared to classical detection methods, proteomics and metabolomics do not limit the number of molecules simultaneously analyzed. Proteomic studies using MD samples are technically challenging and the MD membrane pore-size represents one intrinsic limitation. Nonetheless, recovery of substances larger than the formal cut-off value has also been observed in proteomic studies of human brain microdialysate using clinical 20 kDa (42) and research 100 kDa catheters in an experimental study (23). In these studies, proteins larger than the formal cut-off value crossed the dialysis membrane to a significant extent although true concentrations in the extracellular fluid are likely

to be underestimated. One important technical consideration is that it is impossible to use albumin in the perfusate, which is common using high cut-off MD catheters, as albumin masks the other proteins in the proteomic analysis.

Lakshmanan and coworkers developed another possible application of MD/proteomics by comparing microdialysate samples of TBI patients with normal and abnormal metabolism as evidenced by the LPR threshold of 40 (20). They pursued a diagnostic and biomarkers identification approach by combining peptidomics profiling [by matrix-assisted laser desorption/ionization (MALDI)-MS] with classical bottom-up proteomics profiling (LC–MS/MS). They identified differential proteomic changes (i.e., 13 unique proteins) in the cerebral microdialysate of patients with abnormal metabolism as compared to the control group. These proteins consisted of cytoarchitectural proteins, as well as blood breakdown proteins and a few mitochondrial proteins.

Another interesting application of metabolic MD profiling has been performed by Orešič et al. Applying an untargeted metabolomics strategy based on GCxGC TOF-MS platform, microdialysate was analyzed from 12 samples acquired from 4 TBI patients to study the potential relevance of the serum metabolic profiles in TBI to brain metabolism (22). Interestingly, the top ranking serum metabolites associated with TBI were found highly correlated with their MD levels suggesting possible sensitivity to disruption of the BBB. Among these metabolites were sugar derivatives, metabolites related to energy metabolism as well as several hydroxy-acids. Notably, the medium-chain fatty acids (MCFA, C7–C10) were detected at relatively high concentrations in MD as compared to their corresponding concentrations in blood, while the long-chain fatty acids had lower levels in MD than in blood.

MULTIPLEX CHEMICAL PROFILING

While MD is convenient for measuring temporal profiles, its application is typically limited to sampling relatively small panels of markers that can be recovered from the interstitial space. In reality, biochemical changes following TBI are complex and heterogeneous so a more mechanistically complete description requires the use of techniques capable of the sensitive detection of a great number of analytes simultaneously in any particular biosample of interest. Mass spectrometry (MS) is a key analytical platform capable of multiplex analysis of a wide variety of sample types and underpins the emerging technologies of proteomics and metabolomics. It provides quantitative molecular data and can be used either for the untargeted exploratory identification of hundreds of proteins and metabolites or alternatively in a focused way for the quantification of a small number of known molecular features (either proteins or metabolites) with a sensitivity of down to the parts-per-billion level.

A mass spectrometer measures the mass-to charge ratio [*m/z*] of gas-phase ions. Briefly, the sample of interest is first volatilized and ionized in the ion-source. During the ionization process, molecules may break down into a range of smaller fragments with characteristic charges and masses. The resultant ions are then sorted according to their *m/z* ratio by a mass analyzer. Different ionization techniques and mass analyzers are now available with varying cost and performance (43). There are many different modes of MS analysis ranging from semi-quantitative untargeted methods employing high-resolution instruments or quantitative/targeted analysis (44–46). MS instruments may be coupled to liquid phase pre-separation methods such as liquid chromatography (LC-MS), capillary electrophoresis or ion mobility separation to enhance the dynamic range, sensitivity, specificity, and chemical coverage (47–49). Off-line separation techniques (e.g., CAX-PAGE, SDS-PAGE) can be also employed, especially for protein/peptide separation. Given the chemical diversity of proteins and metabolites and the high sensitivity of this technology, MS has proven its superiority in metabolomics and proteomics. Interested readers are referred to several excellent reviews on the basics of MS and its application in *'omics* strategies (50).

Proteomics

Proteomics is the study of the proteome; a set of "all proteins encoded by the genome" (51). Protein expression is highly dynamic, changing in response to environmental stimuli and with the progression of a disease. With advances in technology, neuroproteomics "*a dedicated discipline for the study of the expression, interaction and function of proteins in the nervous system*" has rapidly grown, contributing to the elucidation of the mechanisms of neurological diseases and to the identification of potential biomarkers of injury (52–58). Proteins already identified as having the sensitivity and specificity to be used as clinical biomarkers of TBI include S100B, neuron-specific enolase (NSE), ubiquitin C-terminal hydrolase L1 (UCH-L1), glial fibrillary acid protein (GFAP) myelin basic protein, cleaved tau protein, spectrin breakdown products (SBDPs), and NFL (53, 59–63). Panels of biomarkers may be needed to provide a more accurate and complete description of the pathology (53, 64–66) and could help in patients stratification, selection of treatment strategies, and outcome prediction (53, 56, 67). The application of combined neuroproteomics/neurosystems biology analysis in characterizing dynamic/spatial protein changes and interactions in response to injury is discussed below and cited studies are summarized in **Table 2**. Excellent reviews are available for complete analysis of these aspects (57, 67, 68).

Due to the complex and heterogeneous biology of TBI, the analysis and interpretation of the resultant proteome needs to account for both *spatial and temporal changes*. For example, proteins that increase in abundance within the neocortex may decrease within the hippocampus of controlled cortically injured (CCI) rats (79). Moreover, age-related changes in particular proteins are reported after TBI (79), supportive of "*the vulnerability of older patients and resilience of younger ones in recovery after brain injuries*." Using a label free quantitative platform, Cortes et al. (77) showed that sub-cellular protein translocation and alternative isoform translation occurred in the neocortical tissue from a CCI rat model at 48 h post-injury. They observed the membrane-dissociation of vinculin, a membrane-cytoskeletal protein involved in anchoring actin to the plasma membrane, after injury. Increased cytosolic vinculin might suggest destabilization and retraction of neuronal processes. Furthermore, they

TABLE 2 | MS-based proteomics.

Reference	Patients characteristics	Time (after injury)	Biosample	Techniques employed	Proteomics platform	Key findings
Conti et al. (69)	Severe TBI, GCS < 7 (6 TBI vs 6 controls)	<12 h	CSF	Proteomics	2-DE and MALDI-TOF MS	Upregulation of acute phase response proteins (A1AT, HPT1β, α1/2, and tetramer), presence of FDP
Hanrieder et al. (70)	Severe TBI (3 TBI, no controls)	<9 d	CSF	Proteomics	iTRAQ + nanoflow LC coupled off-line to MS/MS	Temporal profile of protein changes in CSF showing changes in acute phase proteins but also brain specific proteins such as GFAP and NSE
Harish et al. (71)	Mild, moderate, and severe TBI (26 TBI patients, 30 TBI autopsy cases)	<4 d (when available)	Brain tissue (biopsy or autopsy)	Proteomics; electron microscopy; energy metabolism, cytokine, antioxidant, and lipid peroxidation assays; western blot	iTRAQ + SCX LC-MS/MS	Contusional and pericontusional tissues exhibit different proteomic signatures
Hergenroeder et al. (64, 65)	Severe TBI, GCS ≤ 8 (11 TBI vs 11 controls)	<3 d	Serum	Proteomics	iTRAQ + LC-MS/MS	CRP and SAA increase in serum after TBI. In contrast RBP4 was reduced
Sjödin et al. (72)	TBI (2 TBI, no controls)	NA	CSF	Proteomics	ProteoMiner protein enrichment technology based on HLL, OFFGEL isoelectric focusing of tryptic peptides, LC-MS/MS	HLL strategy enriched low abundant protein biomarkers in human CSF and increased the number of detected proteins. Well characterized proteins in TBI, i.e., NSE, GFAP, MBP, CK-B, and S-100β were successfully identified
Xu et al. (73)	Severe TBI, GCS ≤ 8 (12 TBI vs 8 controls)	<3 d	Brain tissue (biopsy or autopsy)	Proteomics, western blot	2-plex TMT labeling and LC-MS/MS	Overexpression of proteins involved in glial cell differentiation, immune regulation and apolipoprotein catalysis in the statin pathway
Yang et al. (74)	Severe TBI, GCS ≤ 8 (11 TBI vs 2 controls)	<8 h	Brain tissue (biopsy-frontal cortex-)	Proteomics	2-DE and MALDI-TOF MS	Temporal changes of overall protein expression in TBI (at <3h, 4–6 h and 6–8 h post-TBI) and controls. Significantly changed proteins were mainly involved in metabolism, protein synthesis and turnover, electron transport, cytoskeleton proteins, signaling transduction, stress response, and cell cycle
Gao et al. (75)	Pediatric iTBI (13 TBI vs controls)	<24 h post hospital admission	CSF	Proteomics, western blot	Two-dimensional DIGE, MALDI-MS and LC-MS/MS	HP levels lower in iTBI compared to non-inflicted TBI. PGDS and CC levels was higher in iTBI compared to non-inflicted TBI
Haqqani et al. (76)	Pediatric severe TBI, GCS ≤8 (6 TBI, no controls)	<8 h post injury	Serum	Proteomics, ELISA	ICAT nanoLC-MS/MS	Differentially expressed proteins involved in inflammation, innate immunity, and early stress/defense response (e.g., Toll receptors, signaling kinases, transcription factors, proteases, protein involved in response to oxidative-stress)
Reference	**Injury model characteristics**	**Time (after injury)**	**Biosample**	**Techniques employed**	**Proteomics platform**	**Key findings**
Cortes et al. (77)	CCI (rat)	2 d	Brain tissue (pericontusional cortex)	Proteomics	2D-LC/ion mobility IMS/orthogonal TOF-MS	Assessment of protein dynamics and traslocations, including vinculin whose cytosolic traslocation suggests destabilization and retraction of neuronal processes
Crawford et al. (78)	CCI (mouse) mild and severe	24 h, 1, or 3 m	Plasma	Proteomics, ELISA	iTRAQ + LC–MS/MS	Modulation of protein functional clusters related to acute phase response, oxidative stress, and lipid metabolism as function of TBI and in response to TBI*APOE genotype

(*Continued*)

TABLE 2 | Continued

Reference	Injury model characteristics	Time (after injury)	Biosample	Techniques employed	Proteomics platform	Key findings
Kobeissy et al. (57)	CCI (rat)	1–7 d	Brain tissue (ipsilateral cortex)	Proteomics	CAX-PAGE and LC–MS/MS	Decreased abundance of MMIF, aconitase, SOD, NF, and CaM. Increased abundance in complement C3, Pin1, elongation factor 2, and PACSIN
Mehan and Strauss (79),	CCI (rat) in aged, young adults, and juveniles	3 d	Brain tissue (parietal cortex and hippocampus)	Proteomics, western blot, behavioral tests	2-DE and MALDI-TOF-MS	Modulation of 15 proteins isoforms in relation to age and injury in cortex after TBI. Among these: Two isoforms of HSP27, which changed with age, were upregulated in response to injury and showed interactions age*injury; BSA was increased in juveniles only and showed an age*injury interaction; ApoE showed an age*injury interaction
Wu et al. (80)	FP (rat)	4 d	Brain tissue (hippocampus)	Proteomics, western blot	^{18}O-water differential labeling and multidimensional tandem LC-MS/MS	Downregulation of 76 proteins at 4 d after TBI mainly related to energy metabolism, oxidative phosphorylation, electron transport chain, calcium signaling and homeostasis. An important downregulation of CANB1 was observed in TBI rats.

Patient characteristics describe the injury severity level according to GCS and the number of patients/controls included in the study. Injury model characteristics describes the experimental TBI model (and species) used and the injury severity (if available). Time after injury illustrates the sampling time point/s or window. Biosample specifies the sample used for the analysis. Techniques employed describe the technique and/or assay used for the analysis. Key findings highlight any specific insights or notable findings in the papers. 2-DE, two-dimensional gel electrophoresis; A1AT, α1 antitrypsin; ADP, adenosine diphosphate; APOE, apolipoprotein E; ATP, adenosine triphosphate; BSA, bovine serum albumin; CaM, calmodulin; CANB1, calcineurin B; CAX-PAGE, cation–anion exchange chromatography-1D SDS gel electrophoresis; CC, cystatin C; CCI, controlled cortical impact; CK-B, creatine kinase B-type; CRP, c-reactive protein; CSF, cerebrospinal fluid; d, day/s; DIGE, difference in gel electrophoresis; ELISA, enzyme-linked immunosorbent assay; FDP, fibrin degradation product; FP, fluid percussion; GFAP, glial fibrillary acid protein; GCS, Glasgow coma scale; h, hour/s; HLL, hexapeptide ligand libraries; HP, haptoglobin; HPT1β and α2/1, haptoglobin 1 β, α2, and α1; HSP27, heat shock protein 27; ICAT, Isotope-Coded Affinity Tag; IMS, ion mobility spectrometry; iTBI, inflicted TBI; iTRAQ, Isobaric Tags for Relative and Absolute Quantitation; LC, liquid chromatography; LC–MS/MS, liquid chromatography-tandem mass spectrometry; m, month/s; MALDI, matrix-assisted laser desorption/ionization; MBP, myelin basic protein; MMIF, macrophage migration inhibitory factor; MS, mass spectrometry; NA, not available; NF, neurofascin; NSE, neuron-specific enolase; PACSIN, protein kinase C and casein kinase substrate in neurons protein 1; PDGS, prostaglandin D2 synthase; Pin1, peptidyl-prolyl cis-trans isomerase A; RBP4, retinol binding protein 4; SAA, serum amyloid A; SCX, strong cation exchange; SDS-PAGE, sodium dodecyl sulphate-polyacrylamide gel electrophoresis; SOD, superoxide dismutase; TBI, traumatic brain injury; TMT, tandem mass tag; TOF, time-of-flight; MS, mass spectrometry.

highlighted the relevance of protein isoforms (e.g., neurofascin) within the TBI-responsive proteome. Wu et al. (80) used a sophisticated stable isotope ^{18}O-water differential labeling and multidimensional LC–MS to profile the proteomic response at 4 days-post TBI in the CA3 sub-region of the rat hippocampus rather than the global hippocampus. They proposed a calcineurin compensatory model conferring protection from extensive TBI-evoked down regulation of energetics and aberrant regulation of proteins responsible of synaptic structures/reorganization, paving the way for the "*identification of novel therapeutic targets for* cognitive rescue in TBI."

Traumatic brain injury pathobiology evolves over time and *biomarker kinetics* after injury is a critical factor in their clinical interpretation (81, 82). Crawford and colleagues used a quantitative proteomic strategy (iTRAQ-LC/MS) to observe a panel of plasma proteins varying in their temporal profile (24 h, 1, and 3 months) in response to the severity of injury in a CCI mouse model of mild and severe brain injury. At 24 h post-injury, proteomic changes were greater in the mild than severe injury group, suggesting a more complex acute molecular response. In addition, there was greater overlap in protein regulation between the time points in the mild group, indicating that different temporal profile of protein involvement can be associated with the nature of severe injury (78). Bioinformatic analysis of modulated proteins identified particular biological modules associated with TBI, mainly related to the acute phase response, oxidative stress, and lipid metabolism.

Kobeissy et al. (57) recently reported an in-depth description of the dynamic changes in global neuroproteome between acute (1 day post-TBI) and subacute (7 days post-TBI) TBI in rats using the cation–anion exchange chromatography-1D SDS gel electrophoresis (CAX-PAGE) LC–MS/MS platform. Importantly the authors employed systems biology strategies to infer time-dependent changes in cellular pathways caused by TBI. Proteins involved in cell migration, mitochondrial damage, neuronal toxicity, and heat shock response were uniquely altered at 24 h post TBI, while others related to regeneration, axon guidance, axonogenesis, cell growth, and differentiation were solely altered at 7 days post-CCI. Of interest among a total of 19 proteins showing increase in abundance in both acute and subacute TBI samples, the C3 complement component showed a progressive increase over time. C3 is acute phase protein with key systemic and brain local immune regulatory functions; it can be easily measured in biofluids and could represent a promising candidate marker in TBI.

The minimal/lack of availability of brain tissue TBI and control specimens is an obstacle to obtaining direct information on the brain injury processes. Below we describe some of the proteomic studies that have analyzed human brain tissue, CSF or plasma in TBI patients to show how biospecimen selection

represents a major challenge in characterizing pathology. Yang et al. (74) investigated how the timing may influence the human brain cortex protein expression profile in 11 patients needing craniotomy. Using a traditional proteomic approach (protein separation by bi-dimensional electrophoresis followed by MALDI-MS-TOF for identification), levels of injury-associated proteins were minimal in the first 3 h after injury, increasing to a maximum at 4–6 h before declining again at 8 h. The functions of the significantly changed proteins were mainly related to metabolism, electron transport, signal transduction, cytoskeletal integrity, stress response, transport, protein synthesis, and turnover. Harish et al. (71) postulated that structural brain characteristics may lead to differences in TBI response and analyzed the proteomic profiles of human contusional and pericontusional tissue (26 individuals) using an advanced technique with improved quantitative accuracy (iTRAQ-LC–MS/MS). The contusional tissue was mainly characterized by altered immune response, and synaptic and mitochondrial dysfunction, while the pericontusional tissue displayed altered regulation of neurogenesis, cytoskeletal architecture, and vesicle proteins. The characteristic signatures of two anatomically adjacent yet distinct regions might help in the mechanistic understanding of injury evaluation. Again, the use of advanced MS-proteomics techniques allowed to identify more than 4,000 proteins in the cerebral parenchyma of post-TBI patients, showing significant alterations in myelin proteins, complement activation, and apolipoproteins (73).

Cerebrospinal fluid and plasma/serum also contain molecular patterns related to the CNS and are alternative biospecimens with higher clinical translational opportunities. CSF is the most representative being in direct contact with the brain particularly in instances where the BBB has been breached. However, protein concentrations vary widely between CSF samples from different TBI patients (55). Plasma/serum samples are easier to acquire than CSF and may have a high overall protein concentration. Furthermore, since blood sampling is safe and well tolerated, normal control data are simple to obtain and such analysis can additionally be applicable to mild TBI where the risks of more invasive techniques are proportionately harder to justify. However, plasma proteins have a wide dynamic range of concentrations, where the presence highly abundant blood-born housekeeping proteins can hinder the discovery of specific proteomic patterns of interest. The analysis of serum proteins is further complicated by extracranial or systemic influences since protein signatures are generally not completely unique to the CNS. Moreover, proteomics patterns found in the CSF are not straightforwardly translatable to blood. Indeed it has been reported that there is only a partial overlap between plasma and CSF proteome (83). One of the first investigations demonstrating the feasibility of a proteomic strategy to study the CSF from TBI patients was performed by Conti et al. (69) using a conventional gel-based proteomic strategy (2-DE and MALDI-TOF-MS) to profile CSF from a small number patients and matched control subjects. Elevated fibrinolysis markers were detected, probably because of blood coagulation after TBI. A similar approach was used to compare the CSF profile from inflicted ($n = 13$) and non-inflicted TBI ($n = 13$) in pediatric patients with severe injury (75). Increased expression of haptoglobin, prostaglandin synthase, and cystatin were found within 24 h of injury. Another study on CSF (72) performed protein enrichment using hexapeptide ligand libraries to reduce protein dynamic range so as to pick-up low abundance CSF proteins. This small proof-of-concept study with only two subjects found proteins associated with degeneration/regeneration, including NSE and GFAP. An interesting time-resolved neuroproteomic study on post-traumatic human ventricular CSF (70) revealed significant temporal changes in the levels of acute phase proteins as well as brain specific proteins (e.g., NSE, GFAP) with a pattern of change between days 3 and 5 indicating sensitivity to secondary events concordant with medical records in a small number of patients. Observations such as these suggest that proteomic analysis may also provide evidence of, and mechanistic insights into, secondary injury. It is noteworthy that the abovementioned CSF proteomic studies also strengthened the use of serum GFAP and NSE as potential prognostic markers for TBI as previously suggested (84, 85).

Although plasma/serum is very clinically convenient, it has been little used in mechanistic-based proteomics studies of TBI. Proteins changes generated in the brain after TBI may potentially migrate into blood but in doing so are greatly diluted. Moreover, plasma and serum proteomics is a challenging task due to the large dynamic range of protein concentration, being 99% of their proteome comprised of 20 highly abundant proteins (86). Despite these hurdles, recent implementation in MS platforms has been essential for the proteomics study of TBI using human plasma/serum.

A feasibility study by Haqqani et al. (76) using isotope-coded affinity tag-based proteomics, identified differences in 95 serum proteins (within 8 h of admission) between six pediatric severe TBI patients and matched controls. These proteins were mainly involved in inflammation and innate immunity indicating a massive defense response. Several of these serum proteins, such as α-spectrin, NSE, tau, and amyloid-A, were likely of brain-origin. In addition, several proteins showed quantitative changes similar to those of S100B, an established serum biomarker of TBI, suggesting that pattern behavior of a group of proteins can help in gaining information about disease characteristics and in the discovery of relevant peripheral TBI biomarkers.

The same technique was used to screen the serum of 11 adult severe TBI patients and matched controls (64, 65). Several proteins altered after injury were in keeping with those identified by Haqqani in pediatric patients, including serum amyloid-A and retinol-binding-protein-4. Interestingly, serum amyloid-A was proposed as indicator of injury severity together with C-reactive protein, whereas retinol-binding-protein-4 was postulated as a predictor of ICP elevation, an important contributor to death/disability following TBI. The authors suggested that possible combination of proteins, each with their own diagnostic window, may help identifying TBI patients who are likely to experience adverse secondary events such as elevation in ICP.

So far, the few clinical proteomics investigations performed on peripheral blood have replicated and strengthened the findings from targeted analysis of specific candidate proteins as TBI biomarkers, recapitulating key features of the neuropathology

seen in humans. However, the novel protein patterns identified in these studies are not yet been validated in independent studies.

Metabolomics

Metabolomics refers to the study of metabolome, which has been defined as "*the complete set of metabolites/low-molecular-weight intermediates (<1000Da), which are context dependent, varying according to the physiology, developmental or pathological state of the cell, tissue, organ or organism*" (87). The technical ability to measure thousands of endogenous metabolites simultaneously as signatures that cellular processes leave behind makes metabolomics an emerging strategy in system biology (88, 89). The application of combined metabolomics/neurosystems biology analysis in characterizing dynamic/spatial metabolic signature in response to injury is discussed below and cited studies summarized in **Table 3**. Hence, metabolomics studies are very promising for understanding complex and multifactorial syndromes and may be a suitable starting point toward personalized medicine.

There are two general analytical approaches to metabolomic analysis. *Targeted* metabolomics refers to the detection and precise quantification (nM, or mg/mL) of a small set of known compounds. It is a hypothesis-driven approach, where then set of metabolites related to one or more pathways is already defined. A limitation of the targeted approach is that it requires the compounds of interest to be known *a priori* and to be available in their purified form. The *untargeted* approach ("metabolite fingerprinting") is instead data driven and is used for complete metabolome comparison (i.e., as many metabolites as possible are measured) (88).

The most common analytical instrumentations used in metabolomics are MS or NMR spectroscopy. Both techniques have advantages and disadvantages, briefly mentioned here and the reader is referred to a number of excellent review articles describing the technology involved (88, 98, 99).

Mass spectrometry is sensitive to ionizable compounds down to the picomolar range. Such high sensitivity makes MS-based methods powerful tools especially in targeted studies when the absolute quantification of groups of metabolites is the goal. In

TABLE 3 | MS-based metabolomics.

Reference	Patients characteristics	Time (after injury)	Biosample	Techniques employed	Proteomics platform	Key findings
Dash et al. (90)	Mild (GSC >12) and severe (GSC <8) TBI (mild TBI, *n* = 20; severe TBI, *n* = 20; healthy volunteers *n* = 20)	<24 h	Plasma	MS-based metabolomics, ELISA	LC–MS and GC-MS	Levels of methionine, SAM, betaine, and 2-methylglycine lower in TBI patients compared to controls, indicating decreased metabolism through the transmethylation cycle. Precursors for generation of glutathione, cysteine and glycine also found to be decreased as were intermediate metabolites of the gamma-glutamyl cycle (gamma-glutamyl amino acids and 5-oxoproline). In mild TBI patients, levels of methionine, a-ketobutyrate, 2 hydroxybutyrate and glycine decreased, albeit to lesser degrees than detected in the severe TBI group
Emmerich et al. (91)	Soldiers with mild TBI (*n* = 21), PTSD (*n* = 34), TBI + PTSD (*n* = 13) and healthy controls (*n* = 52)	Chronic time point	Whole blood, plasma	Genotyping APOE, MS-based lipidomics	LC–MS/MS	PL levels decreased in TBI, PSD (moderate-to-severe) and TBI + PSD compared to controls. MUFA-containing PC and PI species decreased in TBI and TBI + PTSD groups but not in PTSD subjects, ether PC levels were lower in PTSD and TBI + PTSD compared to controls. Within PC and PE classes, ratio of AA- to DHA-containing species decreased in mTBI. APOE ε4 + subjects exhibited higher PL levels
Jeter et al. (92)	Mild (GCS >12) and severe (GCS ≤8) TBI (mild TBI *n* = 18, severe TBI *n* = 20; healthy volunteers *n* = 20, orthopedic injury without TBI *n* = 15)	<24 h	Plasma	MS-based target metabolomics	LC–MS and GC-MS	Plasma levels of arginine, citrulline, ornithine, and hydroxyproline decreased in severe TBI compared to mild TBI or orthopedic injury. Levels of plasma creatine increased in severe TBI compared to healthy and orthopedic injury subjects. Creatine lower in severe TBI patients that developed high ICP compared to those who did not
Jeter et al. (60)	Mild (GCS <12) and severe (GCS ≤8) TBI	<24 h	Plasma	MS-based metabolomics	LC–MS and GC-MS	Levels of BCAAs (valine, isoleucine, and leucine) decreased in TBI compared to healthy volunteers and patients with orthopedic injury. Only plasma levels of methylglutarylcarnitine were increased after severe TBI. BCAAs plasma levels were similar in mild TBI and orthopedic patients but lower compared to healthy volunteers

(*Continued*)

TABLE 3 | Continued

Reference	Patients characteristics	Time (after injury)	Biosample	Techniques employed	Proteomics platform	Key findings
Orešič et al. (22)	TBI, GCS ≤8 (5 TBI, no controls)	Acutely after TBI	ECF, serum	100 kDa MD and MS-based metabolomics	GC × GC-TOF-MS	Two medium-chain fatty acids (decanoic and octanoic acids) and sugar derivatives including 2,3-bisphosphoglyceric acid are strongly associated with TBI severity. Serum metabolic profile also reflected in brain ECF (MD samples). Top ranking serum metabolites associated with TBI were found highly correlated with their MD levels suggesting possible sensitivity to BBB damage, as well as protective response and altered metabolism post-TBI
Yi et al. (93)	Moderate to severe TBI (72 TBI patients with cognitive deficits, 31 TBI patients without cognitive deficits, 67 healthy controls)	<12 h	Serum	MS-based metabolomics	GC-MS	A serum metabolites panel consisting of serine, pyroglutamic acid, phenylalanine, galactose, palmitic acid, arachidonic acid, linoleic acid, citric acid, and 2,3,4-trihydroxybutyrate was identified to be able to discriminate between TBI patients with cognitive impairment, TBI patients without cognitive impairment and healthy controls
Reference	**Injury model characteristics**	**Time (after injury)**	**Biosample**	**Techniques employed**	**Proteomics platform**	**Key findings**
Abdullah et al. (94)	Severe CCI (mouse)	3 m	Hippocampus, cortex, cerebellum (left and right) and plasma	Behavioral tests, MS-based lipidomics	LC-MS/MS	Total PC-, SM-, and PE-species increased in hippocampus but decreased in cortex and cerebella of TBI mice compared to controls. Total PL levels decreased in plasma of TBI mice. Ether-PC in the cerebella and ether-PE in cortex decreased in TBI mice. PUFA-containing PC and PE species, particularly ratios of DHA to arachidonic acid decreased in the hippocampi, cortex, and plasma of TBI mice
Bahado-Singh et al. (95)	WDI (mouse)	4 h and 1 d	Serum	Target quantitative metabolomics	Biocrates platform with FIA and LC–MS/MS	Thirty-six of 150 measured metabolites were different in TBI compared to control mice. Temporal changes (from 4 to 24 h) were observed in 56 metabolites after TBI. The combination of six metabolites achieved complete accuracy for distinguishing early TBI (4 h) from late TBI (24 h) with spermidine as the most discriminating biomarker. Affected pathway included arginine, proline, glutathione, cysteine, and sphingolipid metabolism pathways
Emmerich et al. (96)	CHI (mouse)	1 d; 3, 6 m; 1 and 2 y	Plasma	MS-based lipidomics, ELISA	HILIC LC-MS/MS	PC, PE, PI, and SM levels decreased with aging. PC, LPC, PE, LPE and PI (but not SM) were decreased at 3 months post-TBI, and all classes were decreased at 24 months post-TBI compared to controls. Total lipid peroxidation was elevated at 3 months post-TBI compared to control when PUFA levels were decreased.
Sheth et al. (97)	mild and severe TBI (rat), tMCAo (mouse), acute stroke (9 stroke patients, 5 stroke-mimic patients)	TBI and tMCAo: 4 h, 1, 2, and 7 d. Stroke patients: within 3 h from the first symptom	brain tissue (only mice and rats), plasma, sphingolipids extraction	TTC, immunostaining, MRI, target MS-based lipidomics	LC-MS/MS	*TBI*: 56 SLs species were present at higher concentration in brain compared to plasma. SL increased in plasma from 6 to 24 h post TBI with SM increase proportional to CCI severity. *tMCAo*: 45 SLs species were present at higher concentration in brain compared to plasma. SL increased in plasma from 6 to 24 h post tMCAo with SM and Cer showing the largest relative changes. SM + Cer defined the SL score. *Stroke patients*: within 3 h post-stroke, SL score was higher than in stroke-mimic patients. The SL score correlated with the volume of ischemic brain tissue by MRI

Patient characteristics describe the injury severity level according to GCS, and the number of patients/controls included in the study. Injury model characteristics describe the experimental TBI model used and the injury severity (if available). Time after injury illustrates the sampling time point/s or window. Biosample specifies the sample used for the analysis. Techniques employed describe the technique and/or assay used for the analysis. Key findings highlight any specific insights or notable findings in the papers.
AA, arachidonic acid; APOE, apolipoprotein E; BBB, blood–brain barrier; BCAAs, branched-chain amino acids; CCI, controlled cortical impact; Cer, Ceramides; CHI, closed head injury; d, day/s; DHA, docosahexaenoic acid; ECF, extracellular fluid; ELISA, enzyme-linked immunosorbent assay; FIA, flow injection analysis; GC, gas chromatography; GCS, Glasgow coma scale; h, hour/s; HILIC, hydrophilic interaction chromatography; ICP, intracranial pressure; LC, liquid chromatography; m, month/s; MD, microdialysis; MRI, magnetic resonance imaging; MS, mass spectrometry; MUFA, mono-unsaturated fatty acids; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PL, phospholipids; PTSD, post-traumatic stress disorder; PUFA, polyunsaturated fatty acid; SAM, S-adenosylmethionine; SLs, sphingolipids; SM, sphingomyelin; TBI, traumatic brain injury; tMCAo, transient middle cerebral artery occlusion; TTC, 2,3,5-triphenyltetrazolium chloride; WDI, weight drop injury; y, year/s.

exploratory approaches, it can be challenging to identify hundreds of metabolites simultaneously with the same efficiency for technical and computational reasons (100). NMR spectroscopy is used to identify and quantify compounds in solution that are MR detectable. Despite being less sensitive than MS (micromolar range), NMR is sample-non-destructive and it is highly reproducible (101).

The study of small molecules that enter biochemical pathways has a long history in TBI research. For example, glucose and lactate dynamics have been studied with respect to TBI outcome, disease progression, and mechanisms (102, 103). What distinguishes contemporary studies is the technologies available for the simultaneous analysis of many metabolites in a given sample. Metabolomics has been applied to the diagnosis/prognosis of many diseases from cancer to diabetes to cardiovascular pathologies (104–106) and is now an active area of research in TBI. In this section, we will focus on most recent TBI metabolomics investigations based on MS technologies applied to preclinical models and clinical settings as well.

Although, in recent years, there has been a much needed influx of TBI metabolomics studies due to various confounding variables (e.g., selection biases, genetics, diet) and practical limitations (e.g., chronic effects of repeated mild TBI and ethical considerations), animal models are still important for the exploration of metabolic signatures of TBI for the development of potentially translatable approaches in both the detection and clinical monitoring of patients.

Since lipids and phospholipids (PLs) play important roles in the structural and functional integrity of neuronal membranes, vesicular trafficking and in neuroinflammatory responses (91, 107–109), many investigators have focused in identifying lipid abnormalities associated with TBI ("lipidomics").

Disturbances of lipid profiles in a fluid percussion injury (FPI) rat model of TBI have been known since the 1990s (110) and are a consequence of activating the phospatidylinositol 4,5-bisphospate (PIP2) signal transduction pathway in injured brain. Similar findings were reported for another rat CCI model where free fatty acids (FFA) and diacylglycerol (DAG) levels were increased in the sensorimotors cortex and cerebellum of injured rats compared to sham animals (111).

Using a similar model of TBI, Abdullah et al. (94), used LC–MS to investigate the PLs profiles in mice 3 months post-injury and reported decreased ether phosphatidylethanolamines (ePE) levels in the cortices and plasma of injured animals. Moreover polyunsaturated fatty-acid (PUFA)-containing phosphatidylcholine (PC) and PE species were lower in the hippocampus, cortex, and plasma of injured mice, thus indicating that TBI affects both brain and plasma PL levels, with plausible roles in the inflammatory response.

Recent advances in MS techniques have allowed the development of approaches focused on specific lipid subsets permitting in-depth analysis of lipids species (112). By targeting lipid profiling to sphingolipids (SL), Sheth et al. (97) showed large increases in many circulating SL following TBI in rats, with sphingomyelins (SM) being the most prominent species and larger lesions produced proportionately larger increases. Since increases in many SLs species were also noted in plasma of mice after stroke and a linear correlation between circulating SLs and infarct volume was observed in stroke patients with neurological deficits, the authors suggested that lipid subset can be used as biomarkers of brain injury.

Phospholipid abnormalities may persist long after the initial injury, as reported in a closed head injury (CHI) mouse model of TBI (96). By using HILIC LC/MS techniques, it was shown that saturated, mono-unsaturated fatty acids (MUFA) and PUFA were differently regulated over time and ePE species were elevated at 24 h after TBI and decreased relative to controls at chronic stages (3, 6, 12, and 24 months post-injury). Such longitudinal profile could potentially serve not only as surrogate diagnostic marker but also might enable the discovery of molecular targets for precision medicine.

Tracking changes in the serum metabolome to profile ongoing damage was also addressed recently by Bahado-Singh et al. (95) using a targeted quantitative MS platform in a mouse model of TBI. Arginine and proline pathway, glutathione metabolism, SL metabolism, and polyamine pathways changed significantly over time suggesting their potential utility for ascertaining the timing of TBI and for monitoring the degree of oxidative injury and associated neuronal damage.

Clinical evidence of PL involvement in TBI has been available for some time (113, 114), in that increased glycerol (an indicator of PL degradation) concentration is found in the CSF dialysate from brain-injured patients and during the first 24 h in subjects with an unfavorable outcome after severe TBI (115). A cross-sectional study reported that patients with TBI had increased PL within their CSF relative to control subjects (116). Moreover, CSF levels of PC and PE were enhanced in patients who did not survived following severe TBI compared to those who survived (115). Although not really lipidomic studies, they pointed to a possible association between brain PL disturbances and TBI and paved the way to more sophisticated analyses to deep insight the role of lipid classes in TBI pathophysiology.

Detectable PL disturbances in the CSF suggests that such signals might also be detected in peripheral biofluids. In reality, the interplay between brain and systemic lipid metabolism is complex. In fact, the brain can synthesize saturated (SFA) and MUFA containing PL species; whereas PUFA are transported from blood to brain, where they serve many functions (e.g., membrane repair and lipid mediators) (117).

By using an MS-based lipidomic platform able to qualitatively and qualitatively analyze several 100 PL species and their degree of saturation, Emmerich et al. (91) screened a large cohort of military personnel (120 subjects) and found that PL species profiling, together with APO-E genotyping, was helpful to differentiate mild TBI and post-traumatic disorder, whose diagnosis is often difficult because of overlapping symptomatology.

Interestingly, exploiting a sophisticated metabolomic platform based on 2-dimensional gas chromatography coupled to high resolution MS (GCxGC-TOF-MS) Orešič et al. (22) showed that two MCFAs increased in serum from a large set of TBI patients (144 subjects) and this was strongly associated with the severity of TBI. The accumulation of MCFA might be particularly intriguing due to their role in fatty acid oxidation disorders (118). Thus, MCFA variations may inform on mitochondrial dysfunction in

TBI. Sugar derivatives and hydroxyl acids were also upregulated in the serum of TBI patients, suggestive of BBB disruption and disturbed energetics. Outcome prediction was further improved when the metabolite dataset was combined with clinical and imaging variables.

As expected, changes in several other circulating metabolites may also contribute to the "TBI metabotype." Differences in plasma levels of L-arginine and its key metabolic products have been found between severe and mild TBI patients (92). L-arginine is involved in metabolic pathways critical for cerebral blood flow (CBF) regulation and extracellular matrix (ECM) remodeling (119), thus authors speculated that alteration in circulating arginine found in severe TBI patients may contribute to brain injury such as decreased CBF and collagen synthesis.

Cognitive impairment is one of the most significant TBI-associated disabilities (120). TBI patients with and without cognitive impairment, and healthy controls could be discriminated by a panel of serum metabolites (serine, pyroglutamic acid, phenylalanine, galactose, palmitic acid, arachidonic acid, linoleic acid, citric acid, and 2,3,4-trihydroxybutyrate), identified by GC-MS, although the study was small (93). Nevertheless, post-TBI cognitive impairment was associated with altered metabolism of amino acids, carbohydrates, and lipids. The detrimental role of lipid dyregulation during neurodevelopment and repair after TBI was suggested by the altered levels of arachidonic acid (increased) and palmitic acid (decreased) TBI patients with cognitive impairment (121, 122). Indeed, arachidonic acid, a pro-inflammatory and oxidative lipid species, was also found to be significantly increased in serum from TBI subjects (123, 124) and was associated with platelet dysfunction following TBI (125).

A targeted metabolomic study showed that branched chain amino acids (BCAA) concentration was decreased in TBI patients (60) although this was not confirmed by Orešič et al. (22) probably due to the high variability in BCAA levels. A further targeted metabolomic investigation, based on a combination of LC–MS and GC-MS techniques, reported that severe TBI patients have low levels of plasma methionine and its metabolic products (e.g., S-adenosylmethionine, 2 methylglycine) and their decrease may contribute to brain injury pathology (90). However, caution has to be exercised when considering circulating dietary amino acids such as methionine, when diet intake is not controlled for.

NOVEL SPATIALLY RESOLVED TECHNOLOGIES

The proteomic and metabolomic/lipidomic techniques described thus far rely on global measurements from a particular body compartment. However, chemical changes occur over spatial length scales that can reach down to the sub-cellular level and are dynamic over time. To further understand biochemical events at a local tissue or even cellular level, a spatial mapping is required and imaging plays an important role. Traditionally, this has been achieved via immunohistochemical or other labeling techniques. However, in the context of the '*omics* paradigm presented, such labeling techniques are restricting as they typically probe only a few different species at a time and can only be applied to *ex vivo* tissue sections. A label-free spatially resolved technique yielding pleiotropic chemical information would be highly desirable, particularly if this could be achieved in a non-destructive way that could ultimately be deployed *in vivo*.

Mass Spectrometry Imaging (MSI)

For accurately understanding the pathological condition of a tissue such as brain, which has many functional compartments it is not only necessary to determine the spectrum of molecular features involved in the processes, but also to visualize their spatial distribution within the tissue. MSI is one of the latest rapidly growing techniques for the identification and spatial localization of molecules in tissues (126, 127). MALDI is the most common ionization techniques used in MSI. Ions are produced directly from a tissue slice coated with MALDI matrix and sequential masses are acquired across the tissue surface (128). In a typical MALDI-MSI experiment "*a tissue section is deposited on a steel plate, sprayed with a matrix solution and analysed by MALDI-MSI. The distribution of biomolecules on a tissue section can be readily visualized in two-dimensions, assigning to each pixel the ion intensity specific for the molecule under study*" (129).

Mass spectrometry imaging permits the simultaneous visualization of many types of molecules such as small molecule drugs, metabolites, peptides, proteins, and lipids. Since MSI is a label-free technique that provides the possibility to combine tissue histological data with MS ones it represents a powerful tool for visualizing simultaneously the distribution of molecules in relation to tissue heterogeneity and its pathological status (130).

Experimental TBI studies revealed that the cortex, hippocampus, and thalamus are selectively vulnerable to injury (131–134). Previous studies reported alterations in the lipid profile as an important contributor to this vulnerability and the evolution of secondary damage in TBI (135–137). Consequently, efforts have been made in determining spatial distribution of altered lipid profile in response to brain injury using MSI.

In a study assessing lipid changes in response to blast-induced mild TBI, major increases in ganglioside GM2 were noted in the hippocampus, thalamus, and hypothalamus after a single blast exposure (138). A concomitant decrease in ceramides was also noted in the same study. Hankin et al. (139) showed changes in lysophosphatidylcholine, PC, PE, and SM that were specific to their adduct ions in response to ischemia/reperfusion injury in the brain. By using an innovative silver nanoparticle protocol, Roux et al. (140) imaged rat brain lipids over time (1, 3, and 7 days), in a CCI model of TBI. Their results indicated that increases in SM and ceramide were already detectable 1 day post-TBI in the injured cortex. In addition, changes in derivatives of DAGs, cholesteryl esters, PE, and PI were noted at later time points (days 3 and 7 post-TBI). The kinetic differences in lipid classes might give an insight into the time-course of injury response and remodeling of injured brain tissue.

A biochemical map of critical mitochondrial PL species of cardiolipin (CL) in several anatomical brain regions from a CCI rat model of TBI was recently provided by Sparvero et al. (141). The authors demonstrated that regional/spatial CL decrease occurred not only within the contusion but also in the hippocampal and thalamic regions, distant from the site of injury. They proposed that the specific decreases in CL, which will ultimately influence

mitochondrial efficiency, "*may constitute an upstream mechanism for CL-driven signalling in different brain regions as an early response mechanism and may underlie the bioenergetic changes after TBI.*"

Optical Spectroscopy

Optical spectroscopy reveals a chemical fingerprint of any given sample, based on specific absorption of different colors of light by different molecular bonds. Analogous to masses held together by springs, energy absorbed from incident light by molecular bonds can generate vibrations and rotations, such as stretching, torsion, or bending, depending on the molecule. The frequencies of such "modes" of oscillation are determined by the nature of the interatomic forces and are, therefore, characteristic to the bond. The frequencies and geometries of permissible modes are governed by quantum mechanical considerations and are dependent on the detailed electronic configurations and symmetry of the bonds in question. Cellular components such as lipids, proteins, and nucleic acids consist of many bonds and, thus, as a whole, provide a rich chemical fingerprint, particularly for frequencies in the infra-red (IR) part of the electromagnetic spectrum. Optical spectroscopic techniques are advantageous for chemical analysis as they are non-destructive; can be easily integrated with imaging; and, in principle, can be easily translated to *in vivo* applications.

Raman spectroscopy (RS) provides a chemical fingerprint based solely on vibrational interactions with tissue. The chemical information obtained from RS is similar to that obtained by IR spectroscopy (although symmetry and charge considerations mean that not all Raman active modes are IR active and *vice versa*). The Raman effect is very weak but high sensitivity can be achieved locally by focusing the probe beam via a microscope arrangement onto the sample being analyzed and using high sensitivity detection instrumentation. The relatively low-cost availability of stable and compact high intensity laser light sources has greatly facilitated this technique. However, heating of the sample limits the signal to noise improvements that can be achieved by simply increasing the incident laser power for biological specimens.

Raman spectroscopy has been applied to models of brain injury from radiation (142) and penetrating trauma (143) as well as in a model of peripheral nerve injury (144). More recently, RS has been used to characterize chemical progression and resolution in a model of focal TBI (145). Raman spectra were recorded from various regions in the cortex in contusional and pericontusional tissue as well as from the contralateral hemisphere in specimens taken at 2 and 7 days post injury as well as from sham controls. Significant differences in chemical spectra were seen. Most significantly, there were differences in protein signal, including strong changes from heme associated with acute hemorrhage at the contusion site at 2 days. Hemorrhagic conversion is an important and devastating process, and one that may directly contribute to cytotoxic and oxidative damage (146). This heme signal was found to have resolved by 7 days consistent with the phagocytic clearance of hemoglobin and heme by macrophages/microglia. Furthermore, the authors found differences in lipid composition—in particular an elevation in cholesterol signal relative to PL at both 2 and 7 days at the site of the contusion and in pericontusional tissue. This lipid sensitivity of RS is particularly attractive given the importance of lipid metabolism in neuronal degeneration and repair and, in particular, the upregulation of cholesterol transport after injury since this material is critical for cellular repair. The ratio of particular Raman peaks relating to the relative concentration of β-sheet protein has previously been suggested as being sensitive to amyloid and has been studied in "cleaner" systems such as the eye lens (147). The authors observed changes in this signal after TBI also. However, there is generally substantial overlap between lipid and protein signals, which can make these observations difficult to interpret with certainty. Acquisition of spectra from pure reference compounds can reduce uncertainty and assist with identification of some specific lipid and protein contributions, such as those described above. Furthermore, overall changes in lipid and protein concentration are easily detectable and relevant to the disease biology. This lipid and protein sensitivity makes RS a promising technology for research into key pathways of interest after TBI. A key advantage of RS is the potential for imaging and it is possible to demonstrate spatial changes in both protein and lipid chemistry.

While RS is a multiplexed technique, the related technique of coherent anti-stokes Raman scattering (CARS) allows the targeted examination and imaging of particular Raman bands with high sensitivity and has been employed in areas allied to TBI, such as the study of myelin degradation processes where it has revealed a calcium-dependent pathway in a spinal cord model of demyelination (148).

Raman spectroscopy is a label-free and non-destructive technique. RS and allied techniques such as CARS show considerable promise as both untargeted and targeted chemically sensitive imaging techniques. If the spatial resolution is sacrificed, in principle, it is possible to miniaturize the equipment and perform RS *in vivo* via implanted fiber-optic probes offering the possibility of a continuous multiplex assessment of chemistry. Although the RS signal is weak, it increases with the fourth-power of incident light frequency and so signals using green or blue laser illumination are far stronger and have better signal to noise than can be achieved with conventional red or IR illumination. However, these frequencies can also excite tissue autofluorescence, which interferes with the baseline of the Raman spectrum making quantitative analysis more difficult. Although a nuisance for RS, flavoproteins such as NADH exhibit strong redox-sensitive fluorescence signals offering the possibility of simultaneous interrogation of the status of the electron transport chain.

FUTURE CHALLENGES IN TBI 'OMICS: OPPORTUNITIES FOR SYSTEM BIOLOGY

The flurry of research in TBI '*omics* over the last decade is promising but many issues have to be addressed before deciphering complex molecular patterns that define TBI biochemistry. The limited sample sizes studied to date, differences in sample collection protocols, in outcome measures and limited number of longitudinal clinical designs are hindering the assessment of patient '*omics* trajectories. A better tracking of protein dynamics and range of post-translational signaling events are also needed. The development of multiplexing quantitative targeted proteomics would help in choosing the most specific candidates from the wide range of potential protein biomarkers found in the literature

and in moving into clinical validation and assay development. Such approaches could complement the use of conventional proteins biomarkers assays and provide a new framework to develop and optimize interventions. The analytical technology will need to be further refined if clinical applications are to ultimately be realized as current experimental techniques are too cumbersome for bedside use. Cost is a further consideration: experimental assays are expensive both in terms of equipment and consumables. While this is perhaps less of a limitation in the intensive care unit where treatment costs are already significant, it is a particular problem for applications in mild/moderate TBI and the benefits in terms of treatment would need to be significant in order to be justifiable with current technology. Having said this, bioassay technology is advancing continuously and there are many potential applications of clinical metabolomics and proteomics outside TBI driving advances, so the future landscape is likely to look very different.

While metabolic changes are fundamental to TBI pathophysiology and metabolomic signatures may contain prognostic information, it remains unclear if these metabolic changes are causative or just a consequence of TBI pathophysiology. Moreover, further studies are needed to determine to what extent observed metabolic profiles are brain specific or whether they reflect interactions with systemic changes.

The fast-growing '*omics* domain have been facilitated by advances in analytical technology such as various MS modalities or future optical spectroscopic methods and are generating a massive amount of molecular data, offering the identification and quantification of hundreds of molecules involved in diverse cellular pathways and, therefore, their consequent phenotypes. The complexity of biological systems and large number of variables coupled with the relatively low number of observations (e.g., samples) make integrative analyses challenging. One of the greatest challenges is that of TBI bioinformatics. The development of easy-to-use informatics software was required to leverage genomic data and similar information technology developments will need to take place before proteomic and metabolomic/lipidomic data can be fully exploited. Progress is being made in developing novel analytical techniques (149, 150). However, the challenges are significant as the data are far more complex than is the case for genetics. A paucity of specialized mathematical, statistical, and bioinformatics tools and the need of considerable computational effort hampers rapid progress in the field (151–153).

Recently, Feala and colleagues elegantly addressed this issue in TBI research (53) with the suggestion that existing TBI data sets available from the literature could be exploited. They compiled a list of proteins candidates from the TBI literature and by applying network and pathway analysis, were able to generate candidate new biomarkers. From their integrative network analysis, the protein kinase ABL1 was particularly appealing being already known as tractable target by imatinib in chronic myeloid leukemia.

Functional interpretation of proteins or metabolites usually includes enrichment analysis and pathway analysis that often overlap as both work by comparing significant features, identified by different statistical workflows, to predefined knowledge databases. To this purpose, many tools exist either free (e.g., MetaboAnalyst,) or on the market (e.g., MetaCore™ from Thompson Reuters; IPA from Qiagen) with their own advantages and drawbacks.

One possibility for a truly comprehensive characterization of TBI might consists in simultaneously monitoring the levels of transcripts/proteins/metabolites from the same study population and in combining the resulting multilevel data to infer the dynamics of the underlying biological networks.

Multiomic integration—the full knowledge of the proteome/metabolome and its spatio-temporal evolution after TBI—would be a powerful insight into the underlying pathobiology. However, the goal of omics techniques is not simply to catalog all the transcripts, proteins, and metabolites; rather it is to understand biological mechanisms. To do this, it is necessary to map metabolomic and proteomic data onto known pathways.

The abovementioned software (MetaCore; IPA) and others, such as InCRoMAP (154), 3Omics (155), IMPaAa (156), already allow the mapping of different molecular features (gene, miRNA, protein, metabolite) into biological pathways as networks of interconnected nodes, allowing their visualization for the easy identification of relationships and hidden patterns for hypothesis generation. Recently, network approaches have been used to relate genomic and proteomic signatures in cellular pathways pertinent to experimental TBI-related cellular systems (157).

However, there are still open challenges in '*omics* data integration (including dimensionality, heterogeneity, data source and processing, computational power, and capacity) that need to be faced to take effective advantage of new experimental multilayer data. Moreover, given the complexity of TBI, only a comprehensive and integrated analysis of molecular data with clinical measurements may help to plan an early and appropriate intervention in TBI. In this context, incorporation of '*omics* data into clinical practice may, thus, provides a means to establish new therapeutic targets and to follow individual patient response to therapy. Data mining and machine learning approaches are now representing a very powerful tool since they can be used to develop classification models and to elucidate early multilevel markers signatures which could reveal the biological pathways involved in disease progression (101, 158–160).

Future sophisticated systems biology approaches may allow TBI researchers to make biological sense of the vast quantity of data now available in order to identify novel therapeutic targets and assess the effectiveness of treatments or alternatively, through correlation with population cohorts, to realize a precision medicine approach through the intelligent targeting of interventions to patients in whom they may be most effective.

AUTHOR CONTRIBUTIONS

AE and EZ conceived and coordinated the review group. SM and GV drafted section 1, RP drafted section 2, and SB and JS drafted section 3. All authors read and approved the final manuscript.

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REVIEW
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Metabolomics Profiling As a Diagnostic Tool in Severe Traumatic Brain Injury

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Traumatic brain injury (TBI) is a complex disease with a multifaceted pathophysiology. Impairment of energy metabolism is a key component of secondary insults. This phenomenon is a consequence of multiple potential mechanisms including diffusion hypoxia, mitochondrial failure, and increased energy needs due to systemic trauma responses, seizures, or spreading depolarization. The degree of disturbance in brain metabolism is affected by treatment interventions and reflected in clinical patient outcome. Hence, monitoring of these secondary events in peripheral blood will provide a window into the pathophysiological course of severe TBI. New methods for assessing perturbation of brain metabolism are needed in order to monitor on-going pathophysiological processes and thus facilitate targeted interventions and predict outcome. Circulating metabolites in peripheral blood may serve as sensitive markers of pathological processes in TBI. The levels of these small molecules in blood are less dependent on the integrity of the blood–brain barrier as compared to protein biomarkers. We have recently characterized a specific metabolic profile in serum that is associated with both initial severity and patient outcome of TBI. We found that two medium-chain fatty acids, octanoic and decanoic acids, as well as several sugar derivatives are significantly associated with the severity of TBI. The top ranking peripheral blood metabolites were also highly correlated with their levels in cerebral microdialyzates. Based on the metabolite profile upon admission, we have been able to develop a model that accurately predicts patient outcome. Moreover, metabolomics profiling improved the performance of the well-established clinical prognostication model. In this review, we discuss metabolomics profiling in patients with severe TBI. We present arguments in support of the need for further development and validation of circulating biomarkers of cerebral metabolism and for their use in assessing patients with severe TBI.

Keywords: traumatic brain injury, metabolomics, biomarker, neuromonitoring, outcome, mass spectrometry

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INTRODUCTION

Severe traumatic brain injury (TBI) is usually defined as a brain injury from an external force resulting in Glasgow Coma Scale of 3 to 8 (1) meaning that the patients are primarily unconscious or they gradually became unconscious after the injury. Severe TBI is associated with high mortality (2). About 30% of patients with severe TBI will die and 50% will suffer from at least moderate

disability after 1 year, although some may show excellent recovery (3, 4). The initial severity assessment may be misleading due to frequently occurring confounders (prehospital sedation, hypoxia, inebriation, etc.), and the severity grading may change during the acute injury period, as TBI is a dynamic process with complex and heterogeneous pathophysiology. Early outcome prediction is challenging also due to impending secondary insults.

The primary brain injury results in a complex series of events, which generate a secondary brain injury process. Different insults belonging to a secondary brain injury are aggravated by impairment of energy metabolism that is a consequence of multiple potential mechanisms including both hypoxia and diffusion hypoxia (5, 6), increased non-oxidative processes (7), mitochondrial failure (8), and increased energy needs due to systemic trauma responses, seizures, or spreading depolarization (9, 10). The condition is further convoluted by heterogeneous temporal evolution of brain injury and individual differences between the patients (11, 12). The degree of disturbance of brain metabolism after TBI is also affected by treatment interventions, which are reflected in clinical patient outcome. Although the sustained metabolic crisis in the brain is mostly unsolvable by neurotrauma resuscitation and rigorous intracranial pressure (ICP) control (13–15), the monitoring of secondary brain injury events provides insight into early physiological insults experienced by the brain. It also provides an opportunity to treat physiological disturbances and predict the later pathophysiological course of TBI.

Sedation and neuromuscular blockade in the neurocritical care setting limit the ability of clinicians to obtain a reliable neurologic examination. Additionally, clinically observed deterioration often occurs as a late manifestation of secondary brain injury process. Multimodal neuromonitoring helps to detect, and in many circumstances to treat, cerebral ischemia. By monitoring invasively ICP, brain tissue oxygenation (PbtO2), cerebral perfusion autoregulation with the pressure reactivity index (PRx), and continuous electroencephalography, it is possible to assess and follow the gross physiology within the brain after severe TBI (16). Cerebral microdialysis provides a window into the underlying cellular metabolism of injured neurons by assessing the lactate/pyruvate ratio. An increase in this ratio reflects a decrease in the oxidative mitochondrial metabolism and mitochondrial failure. Thus, the basis for obtaining tissue metabolic data rests on the need for detecting a transition from aerobic to anaerobic metabolism. The switch to anaerobic metabolism is associated with poor neurological outcome in patients with TBI (9, 17, 18).

Cerebral microdialysis can be regarded as a type of local targeted metabolomics study, but there are also other means of assessing a vast spectrum of endogenous compounds with small molecular mass that serve as substrates and intermediates of biochemical pathways in the human body. In the continuing expansion of "omics" in biomedical research, the global study of metabolism at the molecular level, metabolomics, has enabled simultaneous determination of thousands of small molecules at various levels of cellular function due to the advances in systems biology. There is an on-going paradigm shift toward knowledge-based systemic "omics" studies leading to comprehensive metabolite profiling and fingerprint diagnostics in contrast to current hypothesis-driven research (19, 20).

Due to challenges in acute diagnostics, stratification and monitoring of treatment effects of severe TBI, several different methodologies to help the clinician have been studied, including different imaging modalities (13, 21–24), multimodal monitoring of brain and body physiology (9, 16, 25), and different protein biomarkers (26–30). Our current tools give little direct information about the brain's wellbeing, not to mention predicting secondary injuries. Brain-specific or brain-enriched protein biomarkers have been expected to solve these problems, but the vast heterogeneity of TBIs, the variable damage of the blood–brain barrier (BBB), and problems in specificity have prevented them from reaching clinical use, although several studies have shown correlations with outcome (31–35). In this review, we will focus on severe TBI, because its vast complexity poses a special challenge for diagnostics. We discuss the results and prospects of metabolomics to overcome these challenges, as this methodology may be able to offer individual fingerprint characterization of the on-going pathophysiological events, without many problems that face the use of proteins as brain biomarkers.

METABOLOMICS

Clinical Need for New Blood-Based Biomarkers of Severe TBI

Traumatic brain injury has been a clinically challenging problem for several reasons, including poorly understood complex pathophysiology that behaves unpredictably and vast patient and injury heterogeneity. There are a number of sensitive organ-based biomarkers in clinical use for medical emergencies (36) and oncology diagnostics (37). Accordingly, similar markers for TBI have been searched for, in order to assess the nature and severity of the injury and patient outcome (38, 39).

For an ideal universal molecular biomarker of TBI, the compound should be readily measurable in peripheral venous blood or non-invasively collected biological fluid, as diagnostics from cerebrospinal fluid or cerebral microdialyzates are too invasive methods in evaluating mild or moderate TBI. Moreover, severe TBI sets special requirements for biomarkers. For a biomarker to be useful in severe TBI cases, it needs to show changes during the initial stages such as transition to mild cerebral energy crisis or regional swelling. The changes need to be detected prior to the onset of global cerebral energy failure and uncontrollable ICP elevation. Depending on the nature of the diagnostic aim, a biomarker of TBI should be able to confirm the presence or absence of TBI, assess the severity and nature of TBI, monitor treatment effects and predict outcome. Furthermore, validation of a biomarker needs to be linked to established clinically relevant indicators of disease severity, e.g., Glasgow coma scale (1), acute imaging findings (such as acute head computed tomography or magnetic resonance imaging), brain tissue fate as assessed with different methods (40–42), or outcome (43).

It appears highly unlikely that a single biomarker could accurately describe these different clinical needs in a case of severe TBI at the emergency department and intensive care unit. This is because patients and injuries are highly heterogeneous and there is significant uncontrolled variability even within the same category

of TBI severity, merely as assessed by rough clinical measures. In the case of an extremely complex disease, such as severe TBI, the inherent variability needs to be taken into account, because the molecular biomarkers might not be fundamentally related to TBI but rather to normal and reactive physiological processes and protective responses, such as those related to age, gender, diet, CNS comorbidities, and extracranial injuries. Therefore, instead of measuring a single TBI-sensitive biomarker, there is a need for comprehensive injury-sensitive biochemical profiling and individual fingerprint diagnostics.

Challenges in TBI Biomarker Research

Protein-based biomarkers have partially failed to fill the expectations in diagnostics of TBI. The problems have been one-dimensional diagnostic perspectives, sensitivity and specificity for TBI and brain, and the inability to pass an intact BBB. The variability in dysfunction of the BBB as a result of TBI strongly affects the performance of proteins to serve as reliable biomarkers of intracranial events. Small molecules with molecular mass under 1,000 Da are more readily able to pass an intact BBB and are thus much more independent from fluctuating and immeasurable confounders related to BBB dysfunction, which is one of the cornerstones of TBI pathophysiology (44). As metabolic profiling can detect and measure a large number of substances, it may enable accurate characterization and stratification of the TBIs for targeted therapies. Blood-based metabolomics profiling is the preferred method due to practical reasons. Although better brain specificity could be achieved by employing CSF analytics, there is no clinical justification to use CSF for biomarker assessment in cases where it is not necessary.

Intracranial dynamics is efficiently monitored by the current methods such as invasive monitoring of ICP, PRx, and $PbtO_2$, reflecting global and regional changes in patients with severe TBI. The validated methods for monitoring metabolic crisis in the brain following severe TBI have been brain microdialysis, arteriojugular venous differences, and positron emission tomography, of which the first-mentioned is not universally available and the latter is neither universally available nor suitable for patients with unstable or intractable ICP.

Metabolomics As an Opportunity for TBI Diagnostics

Metabolomics is a global approach to study the structure, function, and interactions of low molecular weight metabolites in cells, tissues, and biofluids (45). Unlike in the setting of protein diagnostics, the metabolic profile is a snapshot that provides a window into the *in vivo* enzymatic activity of the brain and body, because free metabolite concentrations affect, and are affected by the global metabolic activity (46, 47). Metabolites can be studied and compared with physiological and pathophysiological conditions, allowing better and more comprehensive understanding of disease processes.

As simultaneous determination of a plethora of molecules has become possible due to the new analytical technologies in systems biology, metabolomics enables a conception of biological organism as a network of interacting cells and their metabolites. Given the highly complex nature of the human brain, metabolomics can be utilized to address the biomolecular interaction networks of the brain in health and disease (20, 48).

Technology and Statistical Methods Used in Metabolomics Diagnostics

Several techniques for metabolomics have been applied in discovery and analysis of different biomarkers. Most methods are based on mass spectrometry (MS), typically combined with chromatographic separation techniques, such as gas or liquid chromatography (GC or LC). Proton nuclear magnetic resonance (^{1}H-NMR) has also been widely applied. The advantage of NMR over MS-based methods is the relative simplicity of the sample preparation required. Additionally, ^{1}H-NMR suffers less from batch-to-batch variation observed in global MS-based approaches. However, because of its poorer sensitivity (micromolar concentration range), it is not so useful for biomarkers of TBI, which are typically present at lower levels (picomolar to nanomolar) in the serum. Another key limitation to ^{1}H-NMR is the resolution of the resultant spectra. Typically, metabolomic profiling occurs on high field systems (600 MHz and above), but there is still significant overlap of the peaks (49). This leads to problems with metabolite identification, even if two-dimensional experiments are performed (50). Furthermore, it makes interpreting the increase in NMR signals difficult as it is often unclear, which metabolite causes the increase in signal if multiple metabolites overlap. On the other hand, MS-based methods have the advantage of being more sensitive than NMR (picomolar-micromolar), which allows for the greater detection of metabolites. This higher number of metabolites allows for the greater coverage of biochemical pathways allowing the mechanisms by which the metabolites are changing to be understood. However, one drawback to MS-based methods is the sample preparation, which is typically based on extraction or protein precipitation, which can crate a source of variation into the analysis. This variation can be corrected by using appropriate class-based standards during the extraction or protein precipitation.

In metabolomics, there are basically two types of methodologies used, namely untargeted and targeted analyses. Untargeted analyses are typically applied in biomarker discovery studies, and they correspondingly aim at analyzing comprehensive metabolic profiles. These methods are usually semi-quantitative, i.e., relative concentrations of metabolites are determined between the study groups. The targeted analyses are generally quantitative, and they are limited to the analysis of specific target metabolites. This is because to fully quantify a metabolite, a standard curve of known concentrations needs to be generated. When profiling all metabolites in a biofluid, it is not possible to have a complete set of pure compounds to generate these standard curves. Therefore, class-based internal standards are used, which allow a relative concentration to be calculated. This is known as semi-quantification. In the more targeted approach where the number of metabolites being analyzed is smaller, it is possible to generate standard curves for all metabolites. An appropriate internal standard to correct for matrix effects during the run can then be used. Matrix effects occur when multiple metabolites elute from

the column at the same time causing ion suppression in the MS (51). Therefore, depending on the sample and its preparation, it is possible to get an apparent reduction in the specific metabolite concentration, which prevents absolute quantitation (52). To minimize these matrix effects requires, the presence of a heavy isotope standard, which elutes at the same time as the metabolite being studied (53). These heavy isotope standards are not always available for the whole metabolome and if they were available, it may be prohibitively expensive.

In an untargeted approach, it is still not possible to analyze the whole metabolome with a single method, because of the large diversity of the metabolites, both in terms of chemical diversity and concentration range. First of all, it is not possible to extract both hydrophilic and hydrophobic metabolites with a single method in a robust manner. In untargeted analysis, the most common approach is to use either GC or LC combined with MS. GC-based systems are suitable for volatile and semi-volatile metabolites, while LC can in principle be used for all types of metabolites. The advantages of GC-based systems are good separation efficiency, capability of analysis of very polar and semi-polar analytes in a single method, and the availability of large commercial mass spectral libraries for the identification of metabolites. The main disadvantage of the GC-based methods is the unsuitability for non-volatile metabolites, and that derivatization is needed for the analysis of polar compounds. The main advantage of the LC–MS methods, on the other hand, is the simpler sample preparation, and the applicability to a wider range of metabolites. However, the LC–MS suffers from matrix effects, which make the quantification more challenging in untargeted methods as compared to GC-based methods. With LC, high-resolution accurate MS systems capable of tandem mass measurements have been most commonly applied for untargeted metabolomic analyses, particularly using quadrupole time-of-flight MS (QTOFMS) and Orbitrap MS systems. With GC, TOFMS and QTOFMS systems are also widely applied for metabolomics, although the simple quadruple MS systems are still the most commonly employed method.

In untargeted metabolomics approaches, raw MS data first need to be processed before it can be analyzed by statistical approaches. Several open source software packages have been developed for this purpose, and MS vendors currently offer their own solutions for metabolomics data processing. Among the open source tools, MZmine (54) and XCMS (55) have been the most commonly applied for LC–MS based approaches. Once the data processing step is complete and the data is made available, e.g., in the matrix format, the metabolic profiles can be studied by a variety of statistical approaches, depending on the experimental setting. The general statistical considerations for metabolomics (56) or any other high-dimensional "omics" data (57) need to be applied. The univariate and multivariate methods applicable to metabolomics/lipidomics data analysis have been reviewed extensively (58). However, to summarize briefly, the use of both univariate and multivariate techniques are required depending on the questions raised in the study. One of the largest problems in metabolomics or lipids is the great number of possible species identified by the analytical technique used. This results in high-dimensional data leading to obscure plots (58). To overcome this issue, multivariate techniques such as principal component analysis are used to group the data. For lipidomic data, clustering techniques can be especially powerful due to the relatively small number of lipid classes (approximately 10) that can be measured in untargeted approaches. Lipids in these classes tend to correlate with each other and change consistently in disease (58). The univariate statistical methods can be very useful in visualizing the data. Heatmaps showing the correlation of metabolites or lipids are often used to show species, which are co-regulated. Box and whisker plots coupled to p-value analysis are used to filter and visualize the sample-to-sample variation within a metabolite of interest. One common pitfall when applying predictive modeling (e.g., for biomarker discovery) from multivariate data is the lack of proper validation. In the literature, the most commonly applied approach for this purpose is the partial least squares discriminant analysis (PLS-DA) (59). However, this approach suffers from so-called overfitting, and the reported models, if developed and tested on the same dataset, tend to be over-optimistic, particularly if improper internal validation is applied. Ideally, the PLS-DA model (or those derived from other multivariate methods) needs to be tested and reported on an independent dataset, which has not been used for the model development. A typical workflow of a metabolomics study is demonstrated in **Figure 1**.

Metabolomics Applied to Diagnostics of TBI

There are three studies that have utilized a lipidomics approach in TBI research: in a murine model (60) and in humans (61, 62), while two studies have employed metabolomics approach in humans with TBI (63, 64). Additionally, there are ^{1}H-NMR metabolomics studies conducted on murine brain tissue specimens and plasma (65), and on human CSF (66). The metabolites that have been significantly associated with TBI in these studies are listed in **Table 1**.

Viant and colleagues studied brain tissue specimens and plasma of rats that were exposed to fluid percussion injury (65). The samples were obtained 1 h after injury. They found decreased levels of ascorbate in cortex and hippocampus, glutamate in the cortex and hippocampus, phosphocholine and glycerophosphocholine in the cortex and hippocampus, and N-acetylaspartate in the cortex and hippocampus. While TBI had an effect on the metabolomic profile found in brain tissue, no clear effects were detected in plasma samples (65).

Glenn and colleagues studied single CSF samples of 44 patients with severe TBI and 13 non-injured control patients with normal pressure hydrocephalus or unruptured intracranial aneurysms (66). The ^{1}H-NMR spectra showed prominent peaks for lactate, acetate, beta-glucose, total creatine, pyruvate, glutamine, alanine, creatinine, alpha-glucose, and a doublet propylene glycol. Patients with severe TBI had significantly higher levels of propylene glycol and lower levels of creatinine than the controls. The timing of CSF draws were not matched with clinical events, but in multivariate model, the profile including propylene glycol, glutamine, alpha-glucose, and creatinine was associated with cerebral metabolic rate of oxygen, ICP, and outcome. The study did not provide any information on statistical values (66).

Daley and colleagues reported that in adolescent male hockey players who had sustained a concussion, a set of metabolites relying

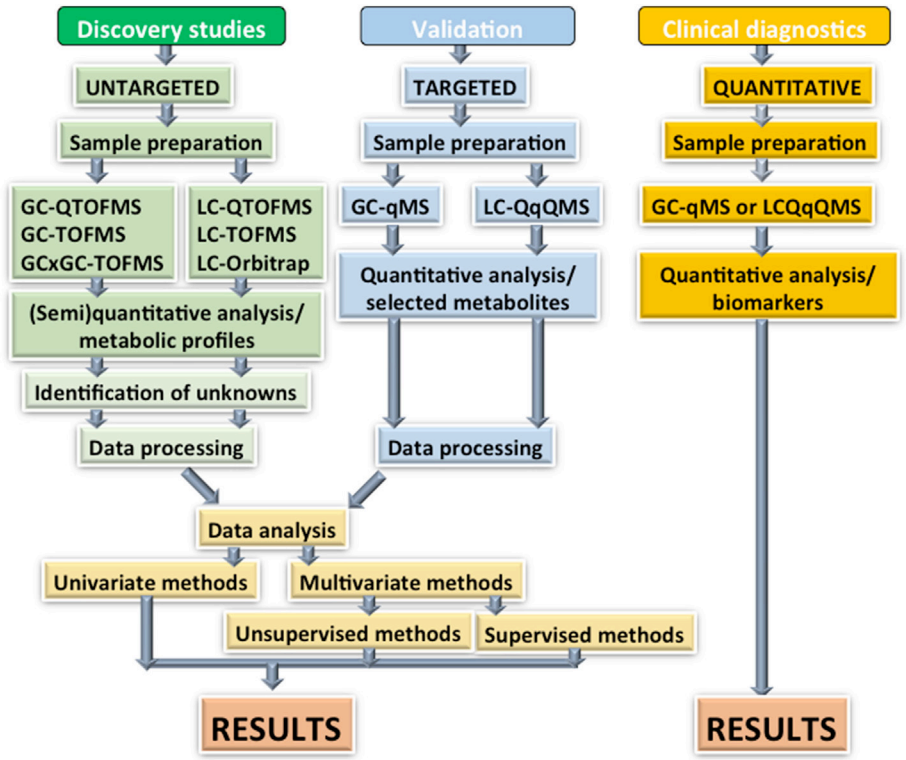

FIGURE 1 | Overview of analytical strategies for metabolomics: there are three key phases required for the development of metabolomics biomarkers if they are to be used in a clinical setting. The initial discovery phase can capture a large number of metabolites using a combination of GC and LC-based techniques. High separation efficiency preferably combined with high mass accuracy is important at this stage due to the high number of metabolites with similar masses. These techniques generate semi-quantitative measurements, which makes them unsuitable for clinical practice. These techniques can also identify unknown compounds, which potentially require time and extra experiments to identify. The validation stage is a key to any biomarker study. Once the metabolites of interest have been identified from the discovery phase, a more targeted method must be developed aimed at only quantifying these. This method needs to be applied to the original data as well as new independent samples to avoid overmodeling. Finally, a fully quantitative assay must be developed for use in the clinic. The analysis needs to be streamlined and user friendly to ensure cost efficiency. Key: GC, gas chromatography; GCxGC, two-dimensional gas chromatography; LC, liquid chromatography; Q, single quadrupole; QqQ, triple quadrupole; TOF, time-of-flight mass spectrometry.

notably on glycerophospholipids accounted for 82% of the variance between 12 concussed and 17 non-concussed athletes. The group utilized ^{1}H-NMR and a method using both direct injection and LC combined with tandem MS. The two methods together cover amino acids, acyl carnitines, specific lipids, and some amines (FIA/LC-MS) as well as glucose, specific hydroxyl acids, and ketone bodies (^{1}H-NMR). The method combining multivariate statistical analysis and machine learning exhibited 92% accuracy rate in diagnosing a concussion (63). However, the possible cofounding factors, such as diet, time from last meal or BMI were not accounted for in the statistical analyses, and the results have not been independently validated in another study group.

TBIcare investigators and Turku Centre for Biotechnology Systems Medicine research group applied comprehensive metabolic profiling of serum samples from two large independent cohorts of patients with full spectrum of TBI and orthopedic injuries (64). Serum metabolomic profiles from 144 patients with mild, moderate or severe TBI were investigated. A control group comprised 28 patients with acute orthopedic injuries without an acute or earlier TBI. The samples were taken upon admission to emergency department (<12 h after the injury). Two-dimensional GC coupled to time-of-flight MS was utilized to analyze the serum samples. The metabolite profiles of the four patient groups were compared to an independent validation cohort from Addenbrooke's Hospital (Cambridge, UK) comprising 67 patients with TBIs of all severities and patients with orthopedic injuries. Decanoic and octanoic acid, which are medium-chain fatty acids, and sugar derivatives including 2,3-bisphosphoglyceric acid were strongly associated with the severity of TBI. These metabolites were detected in significantly higher concentrations in patients with TBI (with or without other injuries) than in patients with orthopedic injuries without any suspicion of CNS trauma. Metabolite levels in patients with mild TBI followed the same pattern as in more severe TBI, but the magnitude of change compared to controls was less than in severe TBI. Brain microdialyzates were also analyzed from 12 samples acquired from patients with severe TBI in the validation cohort, in order to compare the significant serum metabolites with brain extracellular metabolites. The levels of top-ranking serum metabolites associated with TBI correlated highly with their levels in brain microdialyzates, thus suggesting disruption of the BBB. As a second main aim of the study, a prognostic model was developed

TABLE 1 | Metabolites that have been reported to be significantly associated with traumatic brain injury (TBI).

Metabolite name	Metabolite type	Source	Quantity in TBI	Reference
Decanoic acid	Medium-chain fatty acid	Human serum	Upregulated	(64)
Octanoic acid	Medium-chain fatty acid	Human serum	Upregulated	(64)
2,3-bisphosphoglyceric acid	Glyceric acid derivative	Human serum	Upregulated	(64)
Alanine	Amino acid	Human serum	Downregulated	(64)
Serine	Amino acid	Human serum	Downregulated	(64)
Indole-3-propionic acid	Tryptophan deamination product	Human serum	Downregulated	(64)
12 different choline plasmalogens	Glycerophospholipids	Human plasma	N/A	(63)
Acylcarnitine C5	Amino acid	Human plasma	N/A	(63)
Putrescine	Polyamine	Human plasma	N/A	(63)
Formate	Anion	Human plasma	N/A	(63)
Methanol	Alcohol	Human plasma	N/A	(63)
Succinate	Dicarboxylic acid	Human plasma	N/A	(63)
Propylene glycol	Alcohol	Human CSF	Upregulated	(66)
Creatinine	Imidazoline derivative	Human CSF	Downregulated	(46)
Ascorbate	Salt of ascorbic acid	Rat brain	Downregulated	(65)
Glutamate	Amino acid	Rat brain	Downregulated	(65)
Phosphocholine	Choline derivative	Rat brain	Downregulated	(65)
Glycerophosphocholine	Choline derivative	Rat brain	Downregulated	(65)
N-acetylaspartate	Derivative of aspartic acid	Rat brain	Downregulated	(65)

to discriminate patients with favorable (Glasgow Outcome Scale extended 5–8) and unfavorable (Glasgow Outcome Scale extended 1–4) outcome. In the discovery cohort, the performance of the model reached an area under curve (AUC) of 0.90 (95% CI 0.83–0.95) and in validation cohort an AUC of 0.84 (95% CI 0.75–0.89) (**Table 2**). The added value of the prognostic model was studied together with the established CRASH prognostic model (67), consisting of clinical variables. The stand-alone AUC of the CRASH model was 0.74 in the validation cohort. When the top-ranking metabolites (decanoic acid and pentitol-3-desoxy) from prognostic metabolomic model were combined to CRASH model, AUC reached 0.80. The results demonstrate that TBI is associated with a specific metabolic profile, which is exacerbated proportionally to the severity of TBI.

TABLE 2 | Serum metabolites of which levels distinguish between patients with favorable and unfavorable outcome upon admission in a in two independent cohorts of patients (n = 144 and n = 67, respectively) with full spectrum of traumatic brain injury (64).

Metabolite name	Description
Decanoic acid	Medium-chain fatty acid
Octanoic acid	Medium-chain fatty acid
Tryptophan	Alpha-amino acid
Butanal, 2,3,4-trishydroxy-3-methoxy	Sugar derivative
3-Oxobutanoic acid	Beta-keto acid

In addition to the above metabolites, four unknown sugar derivatives, a phenolic metabolite and an unknown amino acid form a model that predicts patient outcomes with AUC of 0.84 (64).

CONCLUDING REMARKS

A new era is emerging in the diagnostics of TBI. There is a paradigm shift toward comprehensive "omics" studies leading to proteomics and metabolomics profiling and fingerprint diagnostics, in contrast to current clinical diagnostics with non-specific and unreliable clinical markers. In the future, a confluence of multi-time-point proteomics and metabolomics diagnostics and advanced imaging studies will highly likely offer more precise stratification and outcome prediction, while individual point-of-care biomarker monitoring of the injured brain will provide means for assessment of intervention efficacy.

At the moment, the number of papers on metabolomics in TBI is small. The identified metabolites associated with TBI are diverse and they have arises from studies that have employed variable methods. Human TBI is in many ways different from experimental TBI models that produce standardized injuries, which never occur in humans. Animal models could be used to evaluate the origin of some metabolites, but otherwise there is no reason to expect why reproducibility between species would be better for metabolomics compared to, e.g., protein biomarkers or pharmacological interventions in TBI, which have given disappointing results from bench to bedside.

The search for clinically relevant TBI fingerprints has just begun. The identified human serum "TBI metabotype" offers a new avenue for the development of next generation diagnostic, prognostic, monitoring and surrogate markers of broad spectrum of TBIs. At the moment, it is impossible to state that this metabotype is brain-specific, but the current results show that the key metabolites are significantly upregulated in patients with TBI as compared to orthopedic controls.

Compared to earlier pursuits in finding a brain- and TBI-specific single compound in blood, the value of metabolomics is in partly avoiding limitations arising from BBB permeability. Metabolomics provides a fingerprint profile of multiple processes, which is important in complex diseases such as TBI. The next steps require explorative studies for different types of injuries at different points in time and correlating the results with clinical and imaging parameters. These tasks will strongly rely on systems medicine approaches and artificial intelligence to interpret the results in different clinical settings. The ultimate challenge lies in validating future metabolite panels for different clinical needs and at variable time points in this vastly heterogeneous patient population.

AUTHOR CONTRIBUTIONS

All authors devised the review article. JP and AD drafted the first versions of the paper with critical contributions from MO, TH, and OT. All authors reviewed, edited, and approved the final version.

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Conflict of Interest Statement: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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REVIEW
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Serial Sampling of Serum Protein Biomarkers for Monitoring Human Traumatic Brain Injury Dynamics: A Systematic Review

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Background: The proteins S100B, neuron-specific enolase (NSE), glial fibrillary acidic protein (GFAP), ubiquitin carboxy-terminal hydrolase L1 (UCH-L1), and neurofilament light (NF-L) have been serially sampled in serum of patients suffering from traumatic brain injury (TBI) in order to assess injury severity and tissue fate. We review the current literature of serum level dynamics of these proteins following TBI and used the term "effective half-life" ($t_{1/2}$) in order to describe the "fall" rate in serum.

Materials and methods: Through searches on EMBASE, Medline, and Scopus, we looked for articles where these proteins had been serially sampled in serum in human TBI. We excluded animal studies, studies with only one presented sample and studies without neuroradiological examinations.

Results: Following screening (10,389 papers), $n = 122$ papers were included. The proteins S100B ($n = 66$) and NSE ($n = 27$) were the two most frequent biomarkers that were serially sampled. For S100B in severe TBI, a majority of studies indicate a $t_{1/2}$ of about 24 h, even if very early sampling in these patients reveals rapid decreases (1–2 h) though possibly of non-cerebral origin. In contrast, the $t_{1/2}$ for NSE is comparably longer, ranging from 48 to 72 h in severe TBI cases. The protein GFAP ($n = 18$) appears to have $t_{1/2}$ of about 24–48 h in severe TBI. The protein UCH-L1 ($n = 9$) presents a $t_{1/2}$ around 7 h in mild TBI and about 10 h in severe. Frequent sampling of these proteins revealed different trajectories with persisting high serum levels, or secondary peaks, in patients with unfavorable outcome or in patients developing secondary detrimental events. Finally, NF-L ($n = 2$) only increased in the few studies available, suggesting a serum availability of >10 days. To date, automated assays are available for S100B and NSE making them faster and more practical to use.

Conclusion: Serial sampling of brain-specific proteins in serum reveals different temporal trajectories that should be acknowledged. Proteins with shorter serum availability, like S100B, may be superior to proteins such as NF-L in detection of secondary harmful events when monitoring patients with TBI.

Keywords: S100B, neuron-specific enolase, glial fibrillary acidic protein, ubiquitin carboxy-terminal hydrolase L1, neurofilament light, serum, biomarkers, traumatic brain injury

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INTRODUCTION

Globally, traumatic brain injury (TBI) is one of the leading causes of death and disability among young adults, and due to sociodemographic changes, it is increasing among the elderly (1–3). TBI consists of two processes: the initial traumatic impact at the scene causing primary damage to the cerebral parenchyma and blood vessels, which can be followed by the onset of detrimental secondary insults (4), characterized by progressive cell death due to inflammation, impaired cerebral blood flow and metabolic function (5). As cells in the central nervous system are injured/compromised or succumb, they either secrete, release, or leak proteins, some of which are relatively enriched in the CNS (6). By measuring these proteins it is possible to assess the extent of cellular injury. Unconscious patients suffering from TBI are often treated in specialized neurointensive care units (NICU) where the goal is to detect, avoid, and treat these secondary insults to optimize cerebral recovery. Implementing measurement of these proteins of tissue fate ("biomarkers") into clinical practice may help in the detection of secondary injury (7, 8).

The most studied TBI biomarker is S100B (9), a predominantly intracellular-, calcium-binding protein present primarily in mature, perivascular astrocytes (10). Other brain-specific proteins that have been extensively studied in TBI include the glycolytic enzyme neuron-specific enolase (NSE) (11); the astrocytic cytoskeleton component glial fibrillary acidic protein (GFAP) (12); ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) involved in the neuronal production of ubiquitin (12) as well as neurofilament light (NF-L), the smallest component of the axonal cytoskeleton (13). Today, S100B is used locally as an early screening tool in the Scandinavian Guidelines in mild and moderate TBI (14), where low levels in serum have been shown to be able to safely exclude the presence of intracranial injury in mild TBI patients and thus obviate the needs for head computed tomography in these cases. However, it has been suggested that one of the limitations with the protein is the relatively short serum elimination half-life (suggested to be as short as 25 min in patients with no ongoing brain injury) (15). Thus, in patients with mild/moderate TBI without pathophysiological processes to cause a sustained release in S100B, delayed sampling may be falsely reassuring and this is reflected in the guidelines, which suggest a cutoff of 6 h after trauma (16). It is becoming increasingly clear that a specific level in serum is of little importance if in the absence of kinetic considerations.

It is not completely clear how these proteins leave the injured brain and enter the blood. Blood–brain barrier (BBB) disruption (17) or release independent of BBB integrity (18) as well as passage through the newly discovered glymphatic system (19) have been suggested as possible routes. Presumably, these proteins are first released in the brain extracellular space, a component difficult to access for repeated sampling (20), before being transported to the cerebral spinal fluid (CSF) [where a passive diffusion from CSF to blood the first 24 h after injury has been suggested (21)] and/or subsequently into serum where it is most easily sampled. However, there are several factors that may influence this transport and thus the availability in serum, including clearance, redistribution, protein stability, and ongoing release from the damaged brain (22). The protein S100B has been shown to have a 100% renal clearance (23), and may thus be affected in patients with renal insufficiency (15, 24, 25). Studies regarding serum clearance for the other biomarkers are scarce, but as they are larger, it is probable that liver metabolism is involved (26).

Thus, the serum concentrations of these biomarkers over time are the net sum of complex wash-in ("leak" from the injured brain) and washout (clearance and elimination from the blood) processes that are variable over time, together creating a profile with an expected peak time and a decay rate. This "fall" rate after peak gives rise to what is here termed the effective half-life ($t_{1/2}$). This process may vary under different conditions and over time in a way that has not yet been properly studied in biomarker research and is distinct from the elimination half-life. Because of this, it will not be possible to present accurate true serum half-lives of these proteins in TBI cohorts. However, composite peak times and biological half-lives can to some extent be grossly estimated from the literature. In this review, we have chosen to focus specifically on these serum trajectories and temporal profiles after TBI.

While there have been several systematic review articles addressing the utility of different biomarkers in detecting injury and predicting outcome (16, 27, 28), there are no studies that have systematically integrated the current knowledge concerning serial sampling of serum biomarkers in brain injured patients, with the goal of suggesting interpretation of levels and estimating peak times and biological half-lives. Understanding the temporal profiles of biomarkers is crucial, as it will provide pertinent information on how to interpret trends. Additionally, current and ongoing studies assessing treatment efficacy (29) as well as multicenter TBI studies such as CENTER-TBI are providing researchers with large cohorts of serial serum samples, where the utility of serial sampling in monitoring secondary events could be assessed (30).

Aim

By systematically and comprehensively reviewing the available literature on serial serum biomarker sampling in human TBI, we wish to assess temporal trajectories in order to better understand serum $t_{1/2}$ of these proteins.

MATERIALS AND METHODS

A systematic review was performed, using the methodology outlined in the Cochrane Handbook for Systematic Reviewers (31). Data were reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (see Data Sheet S1 in Supplementary Material for PRISMA checklist) (32).

Search Question and Population of Interest

The main question posed for this scoping systematic review was as follows: How do serum S100B, NSE, GFAP, UCH-L1, and NF-L levels change with time following TBI? Thus, we aimed to include all studies reporting at least two serum samples of S100B, NSE, GFAP, UCH-L1, or NF-L (respectively) in human TBI. The

primary outcome of interest was serum dynamics, and the resulting effective serum half-life ($t_{1/2}$), over time for these proteins.

Inclusion/Exclusion Criteria

Inclusion Criteria

All studies including human subjects with TBI, any study size, any age category, and reporting at least two serum samples of either/or S100B, NSE, GFAP, UCH-L1, and/or NF-L.

Exclusion Criteria

Animal studies, non-TBI studies, studies without neuroradiological examinations, studies analyzing the biomarker in other bodily compartments than serum, non-English studies (very few available, only one such study $n = 1$ for S100B was found that could otherwise have been potentially included), meeting abstracts, and studies failing to adequately demonstrate data from multiple sampling.

Search Strategies

MEDLINE, EMBASE, and SCOPUS from December 1, 1945 to January 31, 2017 were searched using similar search strategies, which were individualized for each database interface. The search strategy using MEDLINE and EMBASE can be seen in Data Sheet S2 in Supplementary Material, with a similar search strategy utilized for the other database. All possible MESH-terms were used for the different biomarkers (Data Sheet S2 in Supplementary Material). Reference lists of any review articles on this subject were reviewed for any missed relevant studies.

Study Selection

Utilizing two reviewers, a two-step review of all articles returned by our search strategies was performed. First, the reviewers independently (Frederick Adam Zeiler and Eric Peter Thelin) screened titles and abstracts of the returned articles to decide if they met the inclusion criteria. Any meeting abstracts returned by the search strategy were not included in the final review. However, we hand searched the abovementioned databases for any published manuscripts corresponding to these meeting abstracts, prior to discarding them. Second, full texts of the chosen articles were then assessed, to confirm if they met the inclusion criteria and that the primary outcome of interest was reported in the study (Frederick Adam Zeiler and Eric Peter Thelin). Any discrepancies between the two reviewers were resolved by a third reviewer if needed (Adel Helmy or David K. Menon).

Data Extraction

Using a tailored form, data were extracted from the selected articles and stored in an electronic database. Data fields included the following: number of patients, patient demographics [age and injury severity, usually based on Glasgow Coma Scale (GCS) (33)], type of assay used (technique and manufacturer, if available), sampling frequency, trend over time for the specific biomarker (looking at serum data either in tables or figures presented in the articles), estimated temporal profile/serum availability, and any specific notes concerning the serial sampling of the biomarker. As delta values were not provided, to calculate the $t_{1/2}$ the concentration decrease was divided by time. So, if the first concentration following the peak was 0.50 μg/L and a second sample acquired after 12 h was 0.25 μg/L, it would have resulted in a $t_{1/2}$ of 12 h. If the serum concentration initially increased, the decrease following the peak concentration was used (and was commented on in "notes"). If the concentration only increased over time, it was commented on, and no $t_{1/2}$ was calculated. In the case of long sampling frequency, a non-accurate range for the effective serum half-life was noted.

Quality/Bias Assessment

As only three S100B studies and one UCH-L1 study (29–32) investigated serum dynamics over time, a formal bias assessment of all the included studies is not possible. However, as the goal of this review was to produce a systematically conducted review of the available literature on serial sampling of serum biomarkers in TBI, this is not critical. Our main aim was instead to produce a comprehensive overview of the current literature on the topic.

Statistical Analysis

Due to the heterogeneity of the data, both between severity grades of included TBI patients and varying sampling times and assays used, we could not perform a formal meta-analysis of the collected data. However, we did make histograms for the different biomarker studies with a sampling frequency of 24 h or less that indicated an estimate of $t_{1/2}$.

RESULTS

S100B

A search for S100B identified a total of 3,113 manuscripts. Following removal of duplicates and after assessing full manuscripts, 66 articles were deemed eligible for final inclusion (**Figure 1**) and are listed in **Table 1**.

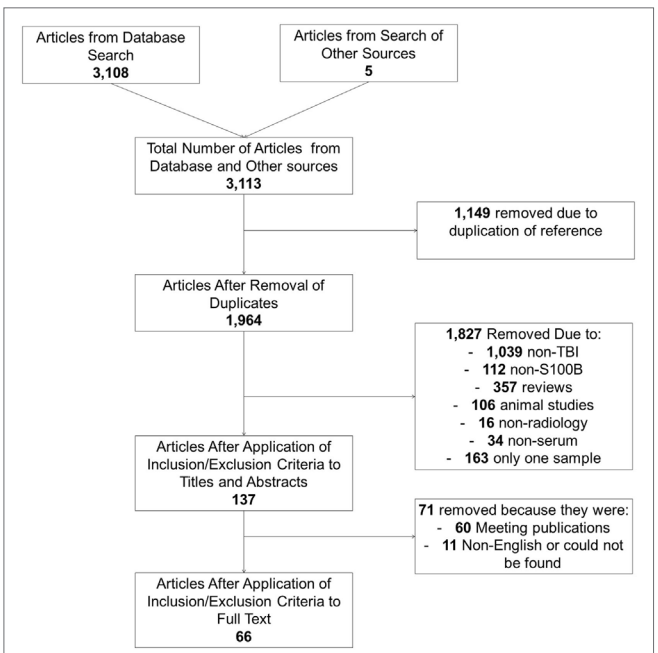

FIGURE 1 | Illustrating the selection of articles with serial sampling of S100B.

TABLE 1 | Analysis of S100B studies.

Reference	Number of patients	Patient characteristics	S100B assay	Sampling frequency	Trend over time	Suggested effective half-life	Notes
Akhtar et al. (62)	17 (7 with TBI)	Pediatric (5–18 years), mild TBI	Liaison, Sangtec	6 h (only two samples)	Decreases first 12 h after trauma	None stated, >6 h	No specific kinetic monitoring. Higher levels in patients with lesions on MRI
Baker et al. (34)	64	Adult, severe TBI patients (GCS < 9)	ELISA, Nanogen Corp.	Initially, 12 h	Decreases first 48 h after trauma, does not reach control levels	None stated, <12 h	No specific kinetic monitoring. Higher levels in patients not treated with hypertonic saline
Berger et al. (67)	100	Pediatric, inflicted and non-inflicted TBI cases. GCS 3–15	ELISA, Nanogen Corp.	12 h	Inflicted TBI longer time-to-peak S100B than non-inflicted TBI	None stated, not enough data to suggest one	Worse GCS have longer time-to-peak
Blyth et al. (68)	10	Adult (39–63 years) mild-to-severe (3–14) TBI	ELISA, Nanogen Corp.	Initially 12 h	S100B levels reach healthy control after 48 h	None stated, 24–48 h	Levels all time below reference levels
Buonora et al. (69)	154 (106 with TBI)	Adult mild-to-severe TBI (GCS 3–15)	TBI 6-Plex, MSD	>48 h	Decreasing quickly over time	None stated, <6 h	No specific outcome concerning biomarker kinetics
Chabok et al. (35)	28	Adult, severe (GCS < 9) diffuse axonal injury TBI	ELISA, CanAg Diagnostics	About 24 h	Decreases quickly over time	None stated, difficult to say, <24 h	Later S100B levels better outcome predictors
Chatfield et al. (70)	20	Adult (16–60 years), moderate-to-severe (GCS 3–11) TBI	LIA-mat, Sangtec	24 h	Decreases over time, reaches control after 5 days	None stated, about 24 h	No specific outcome concerning biomarker kinetics
DeFazio et al. (36)	44	Adult (16–64 years) severe (GCS 3–8) TBI	Unknown	24 h	Decreases the first 72 h	None stated, <24 h	Higher levels in patients with unfavorable outcome
Di Battista et al. (71)	85	Adult moderate-to-severe TBI	Multiplex immunoassay system, MSD	Initially, every 6 h	Quickly declining the first 24 h	None stated, <6 h	Higher levels in patients with unfavorable outcome
Dimopoulou et al. (37)	47	Adult (17–75 years), severe (GCS < 9) TBI	LIA-mat, Sangtec	24 h	Decreases in non-brain dead patients until day 5. Increase in brain dead patients	None stated, 3 days in non-brain dead patients	Higher levels and more volatility in brain dead patients
Elting et al. (72)	10	Adult, moderate-to-severe (GCS 3–13) TBI	LIA-mat, Sangtec	24 h	Decreases the first days, baseline after about 9 days	None stated, about 3 days	No specific outcome concerning biomarker kinetics
Enochsson et al. (63)	19	Adult, mild TBI patients	LIA-mat, Sangtec	4 h (one sample only)	Returns to normal within 4–6 h	4–6 h suggested, looks probable in most patients	No different in kinetics with ethanol in the blood
Ercole et al. (73)	154	Adult, mild-to-severe (GCS 3–15), NICU TBI	CLIA, Liaison, DiaSorin and ECLIA, Elecsys, Roche	12 h	S100B peaks at 27.2 h	None stated, varying over time	Kinetics specifically mapped in patients without secondary peaks of S100B
Ghori et al. (38)	28	Adult (18–65 years), severe (GCS 3–7) TBI	LIA-mat, Sangtec	24 h	Good outcome patients stabilize after 3 days, poor outcome patients increased even after 5 days	None stated, 24 h in patients with good outcome, 72 h in patients with poor outcome	Higher levels in patients with unfavorable outcome
Goyal et al. (21)	80	Adult, severe (GCS < 9) TBI	ELISA, Nanogen Corp.	24 h	Slowly decreasing levels (peak at 24 h), more quickly decrease in patients with good outcome the first 5 days	None stated, about 24 h in patients with favorable outcome and 72 h in patients with unfavorable outcome	Possible to divide patients in trajectory groups where higher levels over time are correlated with an unfavorable outcome

(*Continued*)

TABLE 1 | Continued

Reference	Number of patients	Patient characteristics	S100B assay	Sampling frequency	Trend over time	Suggested effective half-life	Notes
Herrmann et al. (74)	69	Adult (16–67 years) mild-to-severe TBI patients (GCS 3–15)	LIA-mat system, Sangtec	About 24 h	Quickly declining first 12 h, then a plateau until 73 h	None stated, presumably <12 h	Earlier samples better for outcome prediction
Herrmann et al. (75)	66	Adult (16–65 years) mild-to-severe TBI patients (GCS 3–15)	LIA-mat system, Sangtec	24 h	Slowly declining, in some pathologies secondary peaks occurred	None stated, presumably about 24–48 h	Higher in different types of pathologies over time (diffuse axonal injury and edema)
Herrmann et al. (76)	69	Adult (16–65 years) mild-to-severe TBI patients (GCS 3–15)	LIA-mat system, Sangtec	24 h	Relatively slow decline over 96 h	None stated, presumably 49–72 h	Higher area under curve levels in unfavorable outcome. S100B increased 2 weeks and 6 months after injury
Honda et al. (77)	34 (18 TBI patients)	Adult ED TBI patients (GCS 5–14)	ELISA, Yanaihara Institute	24 h	Constantly increased the first 3 days	None stated, presumably >72 h	No specific analysis on biomarker kinetics
Ingebrigtsen et al. (64)	50 (10 patients highlighted)	All ages (6–88 years), mild (GCS 14–15) TBI patients	LIA-mat system, Sangtec	6–12 h (only two samples)	Rapidly decreasing the first 12 h	None stated, <12 h	Early sampled S100B samples decrease rapidly
Ingebrigtsen and Romner (65)	2	All ages (12–73 years), mild (GCS 14–15) TBI patients	LIA-mat system, Sangtec	1 h	Decreasing the first 8 h in patients with injuries on MRI	None stated, about 6 h	Patients with injuries on MRI have elevated S100B levels
Jackson et al. (39)	30	Severe TBI patients	ILA, Byk-Sangtec	3–4 h (only two samples)	Decreasing the first 240 h.	198 min (100 to >500 min presented)	The patients with the highest levels had the most rapid decreases
Joseph et al. (40)	40	Adult (>17 years), severe (GCS < 9) TBI	ELISA, BioVendor	Initially 6, then 18 h	Patients with remote ischemic conditioning decrease over time	None stated, >24 h	No specific analysis on biomarker kinetics
Kellermann et al. (78)	57	Adult, moderate-to-severe TBI	ECLIA, Elecsys, Roche	24 h	Decreasing the first 4–5 days	None stated, about 96 h	No specific analysis on biomarker kinetics. Significant decrease over time
Kleindienst et al. (18)	71	Adult (>17 years), mainly severe TBI	ECLIA, Cobas, Roche	24 h	Steadily decreasing, under reference levels after 20 days	None stated, about 48–72 h	Does not seem to be a kinetic association between CSF and serum
Kleindienst et al. (79)	19	Adult, severe TBI	ECLIA, Elecsys, Roche	24 h	Initial peak to day 2, then decline the first 10 days	None stated, about 96 h	No specific analysis on biomarker kinetics
Korfias et al. (41)	112	Adult (16–86 years), severe (GCS < 9) TBI	LIA-mat system, Sangtec	24 h	Decreasing steadily for survivors, plateau in 96 h. Remaining increased in non-survivors	None stated, about 48 h in survivors and a lot longer in non-survivors	Neurological deterioration during the clinical course is related to increases in S100B
Li et al. (42)	159	Adult (15–71 years) severe (GCS < 9) TBI	ELISA, unknown origin	3 days	Decreases over time, very slow decrease in control group	None stated, 14 days in the treated group, >3 months in the control group	Lower S100B levels over time in the erythropoietin group
McKeating et al. (80)	21	Adult (17–69 years) moderate-to-severe (GCS 3–13) TBI	LIA-mat system, Sangtec	24 h	Decrease over time, up to 48 h, some outliers with increasing levels	None stated, presumably >24–48 h in unaffected patients	More volatility in patients with unfavorable outcome
Mofid et al. (81)	32	Adult, mild-to-moderate TBI	ELISA, BioVendor	24 h and 5 days	Decreasing, and plateauing during 24 h for the progesterone group, while constantly increased for controls	None stated, <24 h in treated patients and >6 days in controls	Lower S100B levels over time in the progesterone group

(*Continued*)

TABLE 1 | Continued

Reference	Number of patients	Patient characteristics	S100B assay	Sampling frequency	Trend over time	Suggested effective half-life	Notes
Murillo-Cabezas et al. (43)	87	Adult (15–76 years), severe (GCS < 9) TBI	ECLIA, Elecsys, Roche	24 h	Decreasing the first 3 days	None stated, 24–48 h	Longer serum half-life in patients with unfavorable outcome
Nirula et al. (82)	16	Adult mild-to-severe TBI	ILA system, Sangtec	24 h	Decrease first 3 days, then stabilizing	None stated, presumably < 24 h	Higher levels in patients with placebo treatment
Nylén et al. (44)	59	All age (8–81 years), severe (GCS < 9) TBI	ELISA, Fujirebio	24 h	Decrease the first 4 days, then plateauing	None stated, 24–48 h	S100BB and S100A1B have slower declines in serum than S100B
Olivecrona et al. (45)	48	Adult (15–63 years), severe (GCS 3–8) TBI	CLIA, Liaison, Sangtec	12 h	Elevated first 4 days, then steep decrease	None stated, presumably about 120 h	Worse correlation between NSE and S100B as time progresses
Olivecrona and Koskinen (46)	48	Adult, severe (GCS < 9) TBI patients	CLIA, Liaison, Sangtec	12 h	Decrease the first 2 days, then stabilizing in ApoE4 groups. Longer time elevated in non-Apo-E4 patients	None stated, about 48 h in Apo-E4 groups, longer in non-Apo-E4 patients	APO-E4 patients have lower S100B levels over time
Olivecrona et al. (47)	48	Adult, severe (GCS < 9) TBI patients	CLIA, Liaison, Sangtec	12 h	Decrease the first three days, then stabilizing	None stated, about 80 h	Later S100B levels better for outcome prediction
Pelinka et al. (83)	79	Adult, mild-to-moderate TBI	LIA-mat system, Sangtec	24 h	Quick decrease for early <12 h samples to 12–36 h. Decrease the first 108 h	None stated, about <12 h	Later S100B levels better for outcome prediction
Pelinka et al. (48)	46	Adult, severe (GCS < 9) TBI	LIA-mat system, Sangtec	24 h	Very high early levels that stabilize after 96 h, especially in multitrauma patients	None stated, 12–24 h	Brain injuries more prolonged release than extracranial trauma
Pelinka et al. (84)	92	Adult, mild-to-severe TBI patients	CLIA, Liaison, Sangtec	24 h	Very high early levels (especially in non-survivors) that stabilize after about 60 h	None stated, 12–24 h	Later S100B levels better for outcome prediction
Petzold et al. (86)	21	Adult, mild-to-severe TBI	ELISA, custom made	24 h	High levels in non-survivors that decrease over time. Little change in survivors that have similar levels as healthy controls after 6 days	None stated, about 72 h for non-survivors	Difficult to compare the levels, are a lot higher than other studies
Petzold et al. (85)	14	Adult (23–56 years), severe TBI	ELISA, custom made	24 h	Slight increase the first day, then a steady decline the first 6 days	None stated, about 6 days	Timing important for S100B interpretation
Piazza et al. (87)	12	Pediatric (1–15 years), mild-to-severe (GCS 3–15) TBI	CLIA, Liaison, Sangtec	48 h (only two samples)	Very heterogeneous trajectories for different patients	None stated, not possible to say	No specific analysis on biomarker kinetics
Pleines et al. (49)	13	Adult (16–67 years), severe TBI (GCS < 9)	ELISA, Sangtec	24 h	Drops relatively quick, "normal" levels after 5 days	None stated, difficult due to log data but probably 48–72 h	No specific analysis on biomarker kinetics
Raabe et al. (50)	15	Adult (19–58 years), severe (GCS < 9)	LIA-mat, Sangtec	24 h	Some patients increase, other steady over time, while many decrease the first 5 days	None stated, difficult to say due to few patients, probably about 48 h in a majority of patients	Patients with secondary increases have a more unfavorable outcome
Raabe and Seifert (51)	3	Adult (17–55 years), severe (GCS < 9) TBI	Unknown	24 h	Secondary increases in three patients	None stated, impossible to say	Secondary increases lead to fatal outcome

(*Continued*)

TABLE 1 | Continued

Reference	Number of patients	Patient characteristics	S100B assay	Sampling frequency	Trend over time	Suggested effective half-life	Notes
Raabe et al. (88)	84	Adult (16–85 years), severe (GCS < 9) TBI	LIA-mat, Sangtec	24 h	Very diverse temporal trajectories in non-surviving patients, steady decline in surviving patients	None stated, difficult to say for non-survivors, probably 24–48 h in survivors	Later samples better for outcome prediction
Raabe and Seifert (89)	25	Adult (18–78 years), severe (GCS < 9) TBI	LIA-mat, Sangtec	24 h	Very dynamic trajectory in patients with unfavorable outcome, steady decline in patients with favorable outcome	None stated, about 72 h in patients with favorable outcome	No specific analysis on biomarker kinetics
Raabe et al. (7)	31	Adult, severe (GCS < 9) TBI patients	CLIA, Liaison, Sangtec	24 h	Increase in TBI patient with cerebral infarction	None stated, difficult to say as only one TBI patient is illustrated	Secondary peaks correlated with a secondary deterioration
Raheja et al. (52)	86	Adult (18–65 years), severe TBI (GCS 4–8)	ELISA, BioVendor	7 days	Decrease the first 7 days	None stated, <7 days	No specific analysis on biomarker kinetics
Rodriguez-Rodriguez et al. (53)	56	Adult, severe TBI (GCS < 9)	ECLIA, Elecsys, Roche	24 h	Steady decline, the first 96 h	None stated, about 24 h	Admission samples worse than 24 h S100B samples for outcome
Rodriguez-Rodriguez et al. (54)	99	Adult, severe TBI (GCS 3–8)	ECLIA, Elecsys, Roche	24 h	Decreasing the first 96 h, greater decrease in patient with better outcome	None stated, presumably 24 h for both survivors and non-survivors	72 h S100B is best for outcome prediction
Rothoerl et al. (55)	15	Adult (17–73 years), severe (GCS < 9) TBI	RIA, Byk-Sangtec	Initially 6, then 24 h	Patients with unfavorable outcome peak at 6 h after admission and then decreasing, favorable outcome patients decrease constantly	None stated, <6 h in patients with favorable outcome and 24 h with unfavorable	No specific analysis on biomarker kinetics
Shahim et al. (56)	72	Adult, severe (GCS < 9) TBI	ECLIA, Cobas, Roche	24 h	Decreases steadily over time (12 days). All normal after 1 year	Not mentioned, 24–48 h	No specific analysis on biomarker kinetics
Shakeri et al. (57)	72	All ages (5–80 years), severe (GCS < 9) TBI	ELISA (?), unknown origin	Initially 48 h	Higher levels in brain dead patients after 48 h than in favorable outcome	None stated, difficult to say due to different sampling.	Highest in patients diagnosed as brain dead
Thelin et al. (11)	417	Adult (>14 years old), mild-to-severe (GCS 3–15) NICU TBI patients	CLIA, Liaison, DiaSorin and Elecsys, Roche	12 h	Decreasing over the first 60 h, faster in patients with favorable outcome. Peaks at about 30 h	None stated, about <6 h initially but longer in later (24 h) samples	S100B influenced by multitrauma first 10 h. 30-h samples best for outcome prediction. More volatility and higher levels in patients with poor outcome
Ucar et al. (58)	48	Severe (GCS < 9) TBI	LIA-mat, Sangtec	48 h	Higher levels on day 3 for the unfavorable group, otherwise unchanged over time	None stated, difficult to suggest one	Patients with unfavorable outcome secondary peaks of S100B
Undén et al. (59)	1	Severe (GCS3) TBI, 22 years old	CLIA, Liaison, Sangtec	Hourly	Very volatile S100B dynamics over time in patient with TBI that succumbs due to cerebral herniation	None stated, difficult to suggest one	Intracranial perfusion necessary for S100B release
Undén et al. (90)	29 TBI	Adult, mild-to-moderate, NICU TBI	CLIA, Liaison, Sangtec	24 h	Elevation > 0.5 μg/L harmful deterioration	None stated, difficult to assess	Strong association between S100B levels and secondary complications
Vajtr et al. (94)	18	Unknown TBI	ECLIA, Elecsys, Roche	>3 days	Decreasing over the first 7–10 days, more so in the less injured group	None stated, probably <3 days.	Decreasing a lot quicker in patients who did not need neurosurgery
Vajtr et al. (95)	38	Different types of presumably adult, severe TBI	ECLIA, Cobas, Roche	>3 days	Decreasing over 1–3 vs 4–10 days in all intracranial pathologies	None stated, <72 h.	Non-expansive contusions highest S100B over time

(*Continued*)

TABLE 1 | Continued

Reference	Number of patients	Patient characteristics	S100B assay	Sampling frequency	Trend over time	Suggested effective half-life	Notes
Walder et al. (91)	49	Severe (AIS > 3, but GCS 3–10), adult TBI	ELISA, Abnova Corp.	Initially 6, then 24 h	Decreases quickly the first 12 h, then more stable	None stated, presumably around 6 h	No difference between multitrauma and non-multitrauma patients. Higher early S100B levels in patients with GCS 3–8
Watt et al. (60)	23	Adult (18–34 years), severe (GCS < 9)	LIA-mat, Sangtec	24 h	Decreases steadily with constant half-life the first 6 days, then leveling	None stated, between 24–48 h	Early samples drawn, quick decline. High early levels associated with an unfavorable outcome
Welch et al. (92)	167	Adult moderate-to-mild TBI (GCS 9–15)	ECLIA, Cobas, Roche	Every 6 h (up to 24 h)	Generally decreasing trends, some increase the first 12 h	None stated, some shorter but seems to <12 h for a majority of patients	After about 8 h, all patients with extracranial injury levels have low levels of S100B
Woertgen et al. (66)	30	Adult (17–73 years), severe (GCS 3–8) TBI	RIA, Byk-Sangtec	Initially 6 h	Decreasing the first hours, then increasing at 24 h with a secondary peak, only to decline later on the first 120 h	None stated, <6 h in early samples but with a secondary increase	Early levels reveal quick early decrease and higher levels in patients with more unfavorable outcome
Yan et al. (61)	42	Adult (16–63 years), severe (GCS < 9) TBI	ELISA, Diasorin	24 h	Steadily decreasing the first 5 days, almost reaching same levels as seen in healthy controls	None stated, 24–48 h	No specific analysis on biomarker kinetics
Zurek and Fedora (93)	63	Pediatric (0–18 years), presumably different severity of injury	ECLIA, Elecsys, Roche	24 h	Steadily declining the first 5 days, some outliers with higher levels	None stated, <24 h for a majority of patients. Some have secondary peaks	Early levels reveal quick early decrease and higher levels in patients with more unfavorable outcome

Number of patients highlighted the total number of patients included in the study, sometimes highlighting in parenthesis how many were actually included with TBI or serial sampling. Patient characteristics described the age groups and injury severity level according to the GCS. The assay described the technique used for the assay and if available the manufacturer. Sampling frequency illustrates with what frequency samples were acquired. Trewnd over time highlights the specific temporal trajectory and dynamics for S100B. Suggested effective serum half-life is noted, as derived from graphs or tables. "Notes" indicate any specific considerations or notable findings of serial sampling in the specific article. TBI, traumatic brain injury; ECLIA, electrochemiluminescent immunoassay; ELISA, enzyme-linked immunosorbent assay; GCS, Glasgow Coma Scale; ED, emergency department; ILA, immunoluminometric assay; RIA, radioimmunoassay; CLIA, chemiluminescent immunoassays; ECLIA, electrochemiluminescent immunoassays; NICU, neurointensive care unit; NSE, neuron-specific enolase; CSF, cerebral spinal fluid.

Patient Characteristics

Generally, the patient characteristics in the S100B studies were severely injured TBI patients (GCS < 9 at admission, unconscious) (21, 34–61) and mild (GCS 14–15) (62–66) or in combination (including moderate GCS 9–13) (7, 11, 18, 67–92) (**Table 1**). All different age groups were analyzed, including only or partial pediatric populations (44, 57, 62, 64, 65, 67, 93), even if a vast majority included solely adult patients (7, 11, 18, 21, 34–38, 40–43, 45–51, 53–56, 59–61, 63, 66, 68, 71–92).

Assays Used to Analyze Serum S100B

The studies used a wide variety of different assays to analyze S100B. Commercial or custom made enzyme-linked immunosorbent assays (ELISAs) were used in some studies (21, 34, 35, 40, 42, 44, 49, 52, 57, 61, 67, 68, 77, 81, 85, 86, 91), as well as other techniques (or not mentioned in the text) (36, 51, 55, 66, 69, 71), even if a majority used clinically available assays such as the (C)LIA-mat system from Sangtec/DiaSorin (7, 11, 18, 37–39, 41, 43, 45–48, 50, 53, 54, 56, 58–60, 62–65, 70, 72–76, 78–80, 82–84, 87–90, 92–95). In general, ELISA samples showed less volatility over time (42, 49, 68, 81, 91). Specifically, they tended to have elevated levels over a prolonged period of time as compared to the automated, clinical assays (42, 49, 52, 77, 81, 85, 86, 91).

Sampling Frequency of S100B

While some studies had more than 24 h between sampling times (42, 52, 57, 58, 81, 87, 94, 95), generally the studies had a sampling frequency of every 24 h (7, 18, 21, 35–38, 41, 43, 44, 48–51, 53, 54, 56, 60, 61, 70, 72, 74–86, 88–90, 93), or sometimes twice daily (11, 34, 45–47, 67, 68, 73), or every 4–6 h (39, 40, 55, 62–64, 66, 69, 71, 91, 92). In contrast to the other biomarkers, there were some studies that assessed S100B hourly in order to track the serum dynamics (59, 65).

Trend of S100B over Time after Trauma

Following trauma, almost all articles proclaim a steadily decline in levels of S100B (18, 21, 35, 36, 40, 43–48, 52–54, 56, 60, 61, 64, 69–72, 78, 80–84, 86, 88, 91, 93–95), while some suggested a slight increase before declining (55, 73, 79, 85, 92). Patients suffering from multitrauma and TBI were seen to have higher levels compared to patients with only TBI (11, 48, 92). Generally, the decreasing trajectory of S100B was strongly correlated with the severity of trauma and/or the outcome for the patient (11, 21,

36–38, 41–43, 50, 54, 55, 58, 60, 62, 65, 66, 71, 75, 84, 86, 93–95). Some papers indicate a very quick decline of serum S100B (39, 49, 83), while some suggest that it is a slower decline over time in relation to when the sample is acquired (11, 60, 64, 69, 71, 73, 74, 85, 93). Several studies indicate volatile S100B trajectories in more unstable patients with detrimental outcomes due to injury development (37, 38, 41, 50, 57, 59, 66, 67, 80, 84, 87–89). Secondary increases ("peaks") of S100B were found in several studies and correlated with secondary adverse events (7, 41, 51, 58, 75, 90). Some clinical trials noted a faster decrease of S100B in serum over time in the trial group as compared to placebo (34, 42, 81, 82).

Suggested Serum $t_{1/2}$ of S100B

Available data suggest that there is a rapid influx and fast $t_{1/2}$ of about 2–6 h in mild TBI patients, which is similar in more severe TBI patients if acquired early (11, 34, 39, 55, 63–66, 69, 71, 74, 83, 91, 92) (**Figure 2A**). Additionally, a slower influx with a later peak and $t_{1/2}$ of about 24 h the first days in severe TBI patients are described (11, 21, 35, 36, 38, 40, 43, 44, 48, 53, 54, 56, 60, 61, 70, 73, 75, 81, 82, 84, 88, 93). Some studies reported $t_{1/2}$ of days (24–120 h) (18, 21, 37, 41, 45–47, 49, 72, 76–80, 85, 86, 89) or even weeks (14 days) (42).

Neuron-Specific Enolase

A search for NSE identified a total of 4,511 manuscripts. Following the removal of duplicates and after assessing full manuscripts, 27 articles were deemed eligible for final inclusion (Figure S1 in Supplementary Material) and are listed in **Table 2**.

Patient Characteristics

Generally, the patient characteristics in the NSE studies were very similar to the S100B studies with a variety of primarily adult, mild/moderate-to-severely (11, 71, 74–77, 80, 82, 96–98), or only severely injured patients (34, 42, 45–47, 49, 52, 54, 61, 66, 99–101) (**Table 2**). However, some looked at more minor injuries and included pe diatric patients (67, 93, 102).

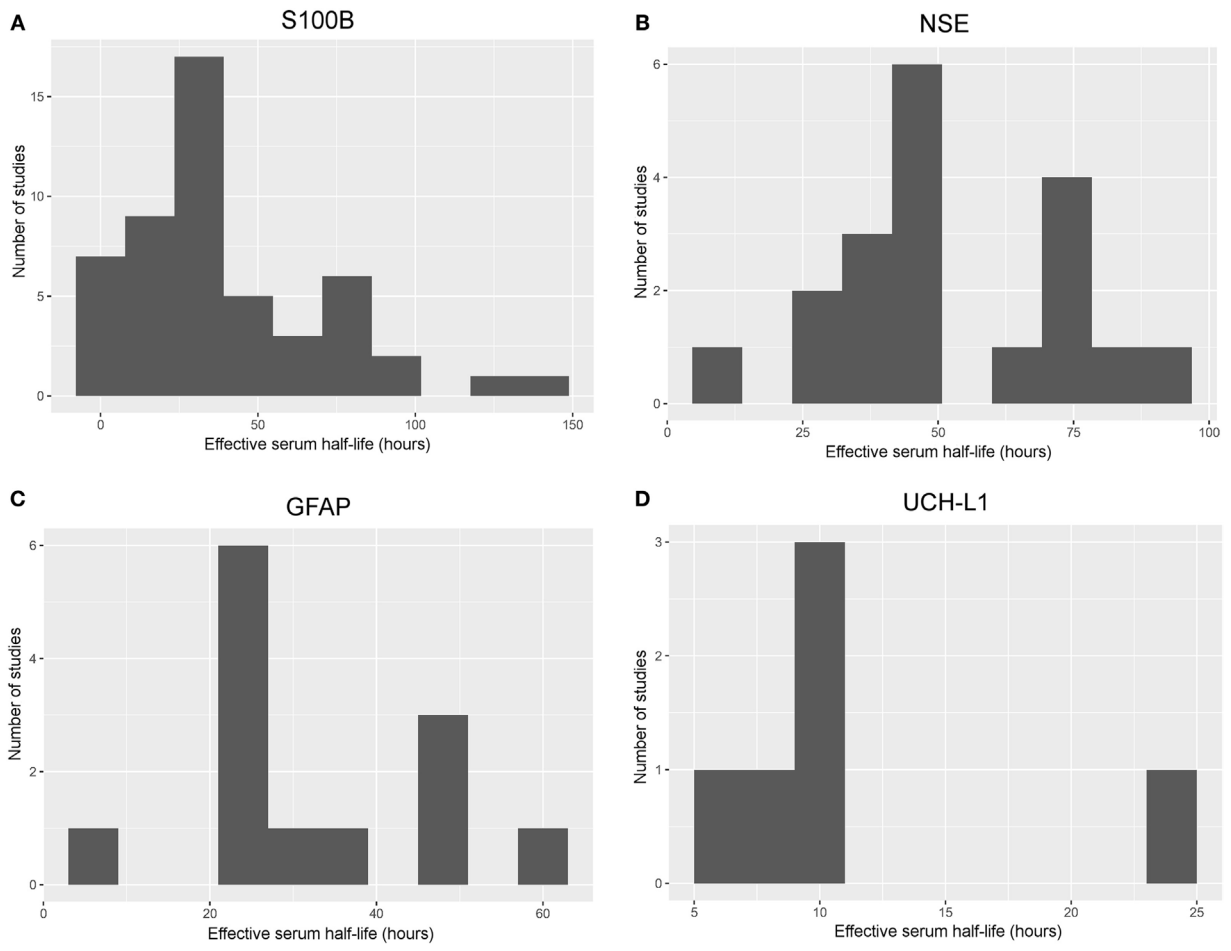

FIGURE 2 | Histograms of frequency of effective serum half-life in different studies. Histograms illustrating the aggregated effective serum half-lives as derived from the different studies including S100B **(A)**, neuron-specific enolase (NSE) **(B)**, glial fibrillary acidic protein (GFAP) **(C)**, and ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) **(D)**. Studies with a sampling frequency of 24 h or shorter and a valid estimate of the effective serum half-life were included. The bin size is set to 10 h in order to easily visualize trends; a relatively short effective serum half-life for S100B and UCH-L1, while it was longer for NSE and GFAP. An effective serum half-life for neurofilament light could not be included as it was impossible to estimate from the available literature.

TABLE 2 | Analysis of NSE studies.

Reference	Number of patients	Patient characteristics	NSE assay	Sampling frequency	Trend over time	Suggested reactive half-life	Notes
Baker et al. (34)	70	Adult, severe TBI patients (GCS < 9)	ELISA, Nanogen Corp.	Initially, 12 h	Decreases quickly after trauma	None stated, 12–15 h the first hours	No specific kinetic monitoring. Higher levels in patients not treated with hypertonic saline
Beers et al. (102)	30	Pediatric TBI (GCS 3–15)	ELISA, Nanogen Corp.	12 h	Increases the first 4 days in inflicted trauma	None stated, not enough data to suggest one	Worse outcome if longer time to peak levels
Berger et al. (67)	100	Pediatric, inflicted, and non-inflicted TBI cases. GCS 3–15	ELISA, Nanogen Corp.	12 h	Inflicted TBI longer time-to-peak NSE than non-inflicted TBI	None stated, not enough data to suggest one	Patients with lower GCS have longer time-to-peak
Buonora et al. (69)	154 (106 with TBI)	Adult mild-to-severe TBI (GCS 3–15)	TBI 6-Plex, MSD	>48 h	Decreasing steadily over time	None stated, about 24 h	No specific outcome concerning biomarker kinetics
Dauberschmidt et al. (99)	9	Severe TBI patients (GCS 4)	RIA	24 h	Steadily increasing in some, unchanged in some, over 10 days	None stated, not enough data to suggest one	No specific outcome concerning biomarker kinetics
Di Battista et al. (71)	85	Adult moderate-to-severe TBI	Multiplex immunoassay system, MSD	Initially, every 6 h	Slowly declining the first 24 h	None stated, probably >24 h (closer to 48 h)	Primary: First 24 h kinetics studied. No difference in NSE levels between outcome
Guzel et al. (96)	169	Mild-to-severe TBI patients	ECLIA, Cobas, Roche	24 h	Declining over time	None stated, presumably close to 48 h for the entire cohort	Slower decline in patients with more severe injuries
Herrmann et al. (74)	69	Adult (16–67 years) mild-to-severe TBI patients (GCS 3–15)	LIA-mat system, Sangtec	About 24 h	Declining over time, stabilizing after 73 h	None stated, presumably 48 h	Later samples not better for outcome prediction
Herrmann et al. (75)	66	Adult (16–65 years) mild-to-severe TBI patients (GCS 3–15)	LIA-mat system, Sangtec	24 h	Slowly declining, in some pathologies secondary peaks occurred	None stated, presumably 73–96 h	Higher in different types of pathologies over time (diffuse axonal injury and edema)
Herrmann et al. (76)	69	Adult (16–65 years) mild-to-severe TBI patients (GCS 3–15)	LIA-mat system, Sangtec	24 h	Slowly declining over 96 h	None stated, presumably 49–72 h	No association between prolonged increases (6 months) of NSE and outcome
Honda et al. (77)	34 (18 TBI patients)	Adult ED TBI patients (GCS 5–14)	ELISA, Alpha Diagnostics	24 h	Constantly increased the first 3 days	None stated, presumably >72 h	No specific analysis on biomarker kinetics
Li et al. (42)	159	Adult (15–71 years) severe (GCS < 9) TBI	ELISA, unknown origin	Initially, 3 days	Decreases over time, very slow decrease in control group not exposed to erythropoietin	None stated, >14 days in the control, 10–14 days in the treated group	Lower NSE levels over time in the erythropoietin group
McKeating et al. (80)	21	Adult (17–69 years) moderate-to-severe (GCS 3–13) TBI	LIA-mat system, Sangtec	24 h	Decrease over time, up to 96 h	None stated, presumably >96 h	More volatility in patients with unfavorable outcome
Nirula et al. (82)	16	Adult mild-to-severe TBI	ILA system, Sangtec	24 h	Decrease first 3 days, then stabilizing	None stated, presumably about 48 h	Higher levels in patients with erythropoietin treatment
Olivecrona et al. (45)	48	Adult (15–63 years), severe (GCS 3–8) TBI	CLIA, Liaison, Sangtec	12 h	Decrease the first 3 days, then stabilizing	None stated, presumably about 72 h	Worse correlation between NSE and S100B as time after trauma increases
Olivecrona and Koskinen (46)	48	Adult, severe (GCS < 9) TBI patients	CLIA, Liaison, Sangtec	12 h	Decrease the first 3 days, then stabilizing	None stated, presumably about 72 h	APO-E4 patients lower NSE levels over time
Olivecrona et al. (47)	48	Adult, severe (GCS < 9) TBI patients	CLIA, Liaison, Sangtec	12 h	Decrease the first 3 days, then stabilizing	30 h is stated in discussion (no reference), but looks more like 72 h	Later NSE levels better for outcome prediction

(*Continued*)

TABLE 2 | Continued

Reference	Number of patients	Patient characteristics	NSE assay	Sampling frequency	Trend over time	Suggested reactive half-life	Notes
Pleines et al. (49)	13	Adult (16–67 years), severe TBI (GCS < 9)	ELISA, Sangtec	24 h	Largely unchanged the first 14 days, slight decrease first day only	None stated, not possible to suggest based on the data	NSE not above reference levels
Raheja et al. (52)	86	Adult (18–65 years), severe TBI (GCS 4–8)	ELISA, DRG International	7 days	Decrease the first 7 days	None stated, <7 days	NSE failed to show any significance to injury over time
Rodriguez-Rodriguez et al. (54)	99	Adult, severe TBI (GCS 3–8)	ECLIA, Elecsys, Roche	24 h	Decreasing the first 96 h, faster decrease with better outcome	None stated, presumably survivors about 24 h and non-survivors about 72 h	48 h NSE is best for outcome prediction
Ross et al. (100)	51 (9 with serial sampling)	Adult, severe TBI	RIA, custom made	Varying frequency (<24 h)	Generally constantly decreasing, one increasing	None stated, probably around 24–48 h, shorter for some	Large spread, some patients have normal NSE levels without any good reason
Shahrokhi et al. (97)	32	Adult (18–60 years), male moderate-to-severe TBI (GCS 3–12)	ELISA, unknown origin	24 h to 6 days	Few samples, decreases over time	None stated, <6 days	No specific analysis on biomarker kinetics
Skogseid et al. (98)	60 (42 mild TBI)	Adult, mild-to-severe TBI	RIA, custom made	Varying frequency, hours (<7 h)	Decreasing the first 12 h in a majority of patients, some steadily low, some increasing	None stated, difficult to assess	Extracranial injury lead to increased levels of NSE
Thelin et al. (11)	417	Adult (>14 years old), mild-to-severe (GCS 3–15) NICU TBI patients	CLIA, Liaison, DiaSorin	12 h	Decreasing over the first 60 h, faster in patients with favorable outcome	None stated, about 24–48 h, longer in patients that died	NSE influenced by multitrauma over time. No specific time frame perfect for outcome prediction. More volatility and higher levels in patients with poor outcome
Vajtr et al. (94)	18	Unknown TBI	ECLIA, Elecsys, Roche	>3 days	Decreasing over the first 7–10 days	None stated, probably 7–10 days	Decreasing quicker in patients who did not need neurosurgery
Woertgen et al. (66)	30	Adult (17–73 years), severe (GCS 3–8) TBI	ELISA, Wallac (maybe with RIA from Sangtec)	Initially 6 h	Decreasing steadily to 24 h, then fluctuating	None stated, 24–48 h	Increasing levels of NSE in patients with high intracranial pressure
Yan et al. (61)	42	Adult (16–63 years), severe (GCS < 9) TBI	ELISA, CanAg Diagnostics	24 h	Steadily decreasing the first 5 days to control levels	None stated, <24 h	No specific analysis on biomarker kinetics
Zhao et al. (101)	128	Adult (16–72 years), severe (GCS < 9) TBI patients with diffuse axonal injury	Unknown	>3 days	Decreasing in the group (magnesium sulfate therapy), while it did not in the placebo group up to 7 days	None stated, > 7 days and even longer in the placebo group	Higher NSE levels in the placebo group
Zurek and Fedora (93)	63	Pediatric (0–18 years), presumably different severity of injury	ECLIA, Elecsys, Roche	24 h	Steadily declining the first 5 days, some outliers with higher levels	None stated, <48 h for a majority of patients. Some have secondary peaks	Higher levels in patients with more unfavorable outcome

Number of patients highlighted the total number of patients included in the study, sometimes highlighting in parenthesis how many were actually included with TBI or serial sampling. Patient characteristics described the age groups and injury severity level according to the GCS. The assay described the technique used for the assay and if available the manufacturer. Sampling frequency illustrates with what frequency samples were acquired. Trend over time highlights the specific temporal trajectory and dynamics for NSE. Suggested effective serum half-life is noted, as derived from graphs or tables. "Notes" indicate any specific considerations or notable findings of serial sampling in the specific article. TBI, traumatic brain injury; ECLIA, electrochemiluminescent immunoassay; ELISA, enzyme-linked immunosorbent; GCS, Glasgow Coma Scale; ED, emergency department; ILA, Immunoluminometric assay; RIA, radioimmunoassay; CLIA, chemiluminescent immunoassays; ECLIA, electrochemiluminescent immunoassays; NSE, neuron-specific enolase.

Assays Used to Analyze Serum NSE

Similar to S100B, the studies used to analyze NSE utilize a wide variety of different assays. A majority of studies used clinically available assays such as the LIA-mat system from Sangtec/DiaSorin (11, 45–47, 74–76, 80, 82) or Elecsys/Cobas systems from Roche (54, 93, 94, 96), but commercial/custom made ELISAs (34, 42, 49, 52, 61, 66, 67, 77, 97, 102) and other techniques (69, 71, 98–101) were also used. Comparable to results

of the S100B, the ELISA methods generally showed higher levels over time and with less dynamics, as compared to the automated assays (42, 49, 52, 97).

Sampling Frequency of NSE

Generally, NSE was sampled either every 6 h (66, 71, 98), 12 h (11, 34, 45–47, 67, 102), and 24 h (54, 61, 74–77, 80, 82, 93, 96, 99, 100) in a majority of studies, while some reported longer sampling frequencies (42, 52, 69, 94, 97, 101).

Trend of NSE over Time after Trauma

Neuron-specific enolase has not been as extensively analyzed as S100B, but it shows similar characteristics with early high levels that decrease over time (34, 61, 69, 94, 97, 98, 100). However, the levels do not seem to decline with the same velocity as S100B (11, 42, 45–47, 49, 52, 69, 71, 74, 76, 80, 82) and even increase without any known association with outcome/injury in a few cases (77, 98, 99, 102). Nevertheless, a slower decline of NSE is seen in patient with more severe injuries or a more unfavorable outcome in many studies (11, 54, 66, 67, 93, 94, 96), and some increasing trajectories in patients with poor outcome were reported (66, 80, 93). Patients with concomitant extracranial injuries had higher levels of NSE (11, 98). Similar to S100B, secondary peaks of NSE were shown in some studies for patients with progressing injuries (75, 93). Likewise, some clinical trials noted a faster decrease of NSE in serum over time in the trial group as compared to placebo (34, 42, 101).

Suggested Serum $t_{1/2}$ of NSE

Available data suggest that the serum $t_{1/2}$ for NSE is longer than for S100B, presumably around 48–72 h in patients with severe TBI (11, 45–47, 54, 66, 71, 74, 76, 82, 93, 96) or even longer (42, 75, 77, 80, 94) (**Figure 2B**). However, some studies reported a shorter $t_{1/2}$ at 12 (34) or 24 h (61, 69, 100).

Glial Fibrillary Acidic Protein

A search for GFAP identified a total of 1,953 manuscripts. Following removal of duplicates and after assessing full manuscripts, 18 articles were deemed eligible for final inclusion (Figure S2 in Supplementary Material) and are listed in **Table 3**.

Patient Characteristics

Similar to studies analyzing S100B and NSE, the patient characteristics of the GFAP patients were mixed, but with a preponderance toward more severely injured patients (12, 52, 71, 77, 84, 95, 103–110), even if milder cohorts also have been analyzed (92, 111, 112). Some studies looked partly, or solely, at pediatric cohorts (12, 113, 114).

Assays Used to Analyze Serum GFAP

A majority of the GFAP studies used various ELISA assays (12, 52, 77, 92, 104–107, 112–114), except two which used an ILA from Liaison™ (84, 108), two studies which used the Randox Biochip™ (109, 110), one an assay from Biotrak™ (95), one used a digital array from Quanterix™ (103), and two that used an immunoassay from MSD™ (71, 111). Currently, there are no clinically available assays. However, fully automated assays are under development.

Sampling Frequency of GFAP

Generally, GFAP was sampled every 24 h (77, 84, 104–110, 113, 114) in a majority of studies (one outlier with 30 days between samples (103)), while some had as short as 6 h sampling (12, 71, 92, 111), and one even 4 h initially (112). Two studies had longer sampling frequencies (52, 95).

Trend of GFAP over Time after Trauma

Similar to the previously studied markers, GFAP seems to decrease after trauma over time (71, 77, 95, 104, 105, 109, 110, 113). However, some studies noted initially increasing levels, up to about 16–24 h following injury (84, 107, 108, 112). GFAP usually remained elevated for a prolonged period of time, as compared with S100B (12, 84). One study showed limited contribution of extracranial trauma (108). As with the other biomarkers, some studies noted prolonged elevated levels, or even continually increasing levels/volatile dynamics, in patients with unfavorable outcome or worse injuries (52, 71, 84, 104, 106–114).

Suggested Serum $t_{1/2}$ for GFAP

The $t_{1/2}$ for GFAP appears longer than for S100B, most studies reported a $t_{1/2}$ at around 24–48 h in severe TBI patients (12, 77, 84, 92, 104, 105, 107–110, 112–114), while some published data suggesting a shorter $t_{1/2}$ (71, 106) (**Figure 2C**).

Ubiquitin Carboxy-Terminal Hydrolase L1

A search for UCH-L1 identified a total of 234 manuscripts. Following removal of duplicates and after assessing full manuscripts, nine articles were deemed eligible for final inclusion (Figure S3 in Supplementary Material) and are listed in **Table 4**.

Patient Characteristics

Generally, the patient characteristics in the UCH-L1 studies were somewhat trichotomized with some of the articles focusing more on the milder TBI spectrum (111, 112), while the others included primarily severe (12, 115–117), or a mix of TBI patients (92, 109, 110) (**Table 4**). No pediatric TBI population was found.

Assays Used to Analyze Serum UCH-L1

Currently, no clinically available assays exist to analyze UCH-L1 and all studies used different ELISAs, either custom made (12, 115–117) or commercially available (92, 112) except for two studies which used a Randox Biochip™ method (109, 110) and one with a ECLIA method from Banyan Biomarkers™ (111).

Sampling Frequency of UCH-L1

In comparison to the other proteins, most UCH-L1 studies had a 4–6 h (12, 92, 111, 112, 115, 116), or 12 h (117), sampling frequency, allowing for a good estimate of the temporal profile. Two studies had a longer and varying sampling frequency (109, 110).

Trend of UCH-L1 over Time after Trauma

In unison with the other markers, UCH-L1 usually decreased steadily following TBI (12, 109, 110, 112, 116, 117). Secondary

TABLE 3 | Analysis of GFAP studies.

Reference	Number of patients	Patient characteristics	GFAP assay	Sampling frequency	Trend over time	Suggested effective half-life	Notes
Bogoslovsky et al. (103)	34	Adult, 21 mild + 13 moderate-to-severe TBI	Digital array, Quanterix	30–60 days	Measured long after trauma, normalized in 30 days	None stated, <30 days (in all patients)	Long-term biokinetics studied. Same GFAP levels as in healthy controls after 30 days
Di Battista et al. (71)	85	Adult moderate-to-severe TBI	Multiplex immunoassay system, MSD	Initially, every 6 h.	Quickly declining GFAP, levels. Staying low after 6 h	None stated, <6 h	First 24 h kinetics studied. Higher GFAP in patients with unfavorable outcome
Fraser et al. (113)	27	Pediatric severe TBI (GCS < 9)	ELISA, R-Biopharm	24 h	Steadily declining. Normalizing on day 10	None stated, 24 h the first days after injury.	First 10 days biokinetics, no monitoring. Higher GFAP in patients with unfavorable outcome
Honda et al. (77)	34 (18 TBI patients)	Adult ED TBI patients (GCS 5–14)	ELISA, BioVendor	24 h	Steadily declining first 3 days	None stated, 48–72 h	No GFAP level difference between diffuse and focal injury
Kou et al. (111)	9	Adult, mild TBI patients	ECLIA, MSD	6 h (up to 24 h)	Decline and increase in two patients	N/A	Worse dynamics in patient with worse white matter injury
Lei et al. (104)	67	Severe TBI patients (GCS 3–8)	ELISA, BioVendor	24 h	Steadily declining first 3 days, then normalizing	None stated, about 48 h	More volatile dynamics in patients with unfavorable outcome
Lumpkins et al. (105)	51 (39 with TBI)	Adult TBI patients	ELISA, BioVendor	24 h	Decreasing, but only samples on day 1 and day 2	<48 h	GFAP levels second day better for outcome prediction. No monitoring aspect
Missler et al. (106)	25	Adult severe TBI (GCS < 7)	ELISA, custom made	24 h	Increasing the first 24 h	None, only increasing, all patients died within 24 h	Plasma and serum levels similar. Suggesting a very short half-life, shorter than for S100B
Mondello et al. (12)	81	Adult (including five pediatric) severe TBI patients, GCS 3–8	ELISA, BioVendor	6 h	Remaining elevated first 24 h after injury	None stated, >24 h	Higher GFAP, with more volatile dynamic, in mass lesions vs diffuse injury
Nylén et al. (107)	59	Adult, severe TBI patients	ELISA, custom made	24 h	Peak after 24 h, decline until 144 h (below reference)	None stated, probably around 24 h	Outcome prediction better for later samples
Papa et al. (112)	325 (35 TBI patients with injuries)	Adult mild-to-moderate TBI (GCS 9–15)	ELISA, Banyan Biomarkers	Initially, every 4 h	Peak after 16 h, decline until 132 h	None stated, probably around 32 h	More volatile dynamics in patients with injuries and requiring intervention
Pelinka et al. (84)	92	Adult mild-to-severe TBI patients	ILA, LIAISON, Sangtec	24 h	Decreasing steadily in non-survivors, peaking 12–36 h after trauma in survivors	None stated, 61–84 h in non-survivors and around 24–48 h in survivors	Later samples better outcome predictor
Pelinka et al. (108)	114	Adult mild-to-severe TBI patients	ILA, LIAISON, DiaSorin	24 h	Similar to Pelinka et al. (84)	Similar to Pelinka et al. (84)	Similar to Pelinka et al. (84)
Posti et al. (109)	324 (71 patients with injury)	Adult mild-to-severe TBI patients	Randox Biochip, Randox Laboratories	Initially, every 24 h	Moderate-to-severe TBI decreasing while mild TBI steady	None stated, moderate-to-severe TBI about 24 h	Early samples best for outcome prediction
Raheja et al. (52)	86	Adult (18–65 years), severe TBI (GCS 4–8)	ELISA, BioVendor	7 days	Patients with favorable outcome decreasing, unfavorable constant the first 7 days	None stated, <7 days probably	Day 7 samples of GFAP had good precision for outcome prediction
Takala et al. (110)	See Posti et al. (109)	See Posti et al. (109)	See Posti et al. (109)	Initially, every 24 h	See Posti et al. (109)	See Posti et al. (109)	See Posti et al. (109)
Vajtr et al. (95)	38	Adult, severe TBI patients	Biotrak Activity Assay System	>3 days	Decrease from 1–3 to 4–10 days	None stated, <10 days	No specific findings related to dynamics, expansive contusions highest levels of GFAP

(*Continued*)

TABLE 3 | Continued

Reference	Number of patients	Patient characteristics	GFAP assay	Sampling frequency	Trend over time	Suggested effective half-life	Notes
Welch et al. (92)	167 (33 patients with injuries)	Adult mild-to-moderate TBI (GCS 9–15)	ELISA, Banyan Biomarkers	Every 6 h (up to 24 h later)	Only increasing the first 24 h	None stated, probably >24 h	Serum concentrations of GFAP less influenced by temporal changes than other biomarkers
Zurek and Fedora (114)	59	Pediatric (0–19 years) severe TBI (GCS < 9).	ELISA, BioVendor	24 h	Generally decreasing the first 3 days, some outliers with dynamic concentrations over time	None stated, probably 24–48 h	Higher levels over time resulted in a general worse outcome

Number of patients highlighted the total number of patients included in the study, sometimes highlighting in parenthesis how many were actually included with TBI or serial sampling. Patient characteristics described the age groups and injury severity level according to the GCS. The assay described the technique used for the assay and if available the manufacturer. Sampling frequency illustrates with what frequency samples were acquired. Trend over time highlights the specific temporal trajectory and dynamics for GFAP. Suggested effective serum half-life is noted, as derived from graphs or tables. "Notes" indicate any specific considerations or notable findings of serial sampling in the specific article. TBI, traumatic brain injury; ECLIA, electrochemiluminescent immunoassay; ELISA, enzyme-linked immunosorbent assay; GCS, Glasgow Coma Scale; ED, emergency department; ILA, immunoluminometric assay; GFAP, glial fibrillary acidic protein.

TABLE 4 | Analysis of UCH-L1 studies.

Reference	Number of patients	Patient characteristics	UCH-L1 Assay	Sampling frequency	Trend over time	Suggested effective half-life	Notes
Blyth et al. (117)	16	Adult ED TBI patients (GCS 3–15)	ELISA, custom made	Every 12 h	Constantly decreasing on group level	None stated, probably about 10 h	Blood–brain barrier assessment with biomarker measurements over time
Brophy et al. (115)	86	Adult severe TBI (GCS 3–8)	ELISA, custom made	Every 6 h	Constantly decreasing on group level	7–9 h	Longer half-life in patients with more severe injury and worse outcome
Kou et al. (111)	9	Adult mild TBI patients	ECLIA, Banyan Biomarkers	Every 6 h (up to 24 h later)	Slight increase in a patient with brain hemorrhage	N/A	GFAP and UCH-L1 are correlated with extent of white matter injury
Mondello et al. (12)	81	Adult severe TBI patients (GCS 3–8)	ELISA, custom made	Every 6 h	Constantly decreasing	None stated, probably about 10–12 h	Focal injuries faster decrease of UCH-L1
Mondello et al. (116)	95	Adult severe TBI patients (GCS 3–8)	ELISA, custom made	Every 6 h	Constantly decreasing on group level, early falls first 12 h	None stated, probably about 10 h	Earlier UCH-L1 levels better for outcome prediction
Papa et al. (112)	325 (35 TBI patients with injuries)	Adult mild-to-moderate TBI (GCS 9–15)	ELISA, Banyan Biomarkers	Initially, every 4 h	Constantly decreasing on group level	None stated, probably 5–7 h first 24 h. Normalized in about 48 h	Slower decrease of UCH-L1 concentrations in patients with hemorrhage and need for intervention
Posti et al. (109)	324 (71 patients with injury)	Adult mild-to-moderate TBI (GCS 3–15)	Randox Biochip, Randox Laboratories	Initially, every 24 h	In severe-to-moderate TBI, decreasing first 3 days, constant in mild TBI	None, difficult to assess from study, <24 h	Earlier samples better accuracy for injury severity than later samples
Takala et al. (110)	See Posti et al. (109)	See Posti et al. (109)	See Posti et al. (109)	Initially, every 24 h	See Posti et al. (109)	See Posti et al. (109)	See Posti et al. (109)
Welch et al. (92)	167 (33 patients with injuries)	Adult mild-to-moderate TBI (GCS 9–15)	ELISA, Banyan Biomarkers	Every 6 h (up to 24 h later)	Serum concentrations in patients with brain injury constant first 12 h, then decreasing	None, many outliers with constant or increasing levels. A peak is seen at 8 h	No specific kinetic analysis other than faster decreasing in non-TBI patients

Number of patients highlighted the total number of patients included in the study, sometimes highlighting in parenthesis how many were actually included with TBI or serial sampling. Patient characteristics described the age groups and injury severity level according to the GCS. The assay described the technique used for the assay and if available the manufacturer. Sampling frequency illustrates with what frequency samples were acquired. Trend over time highlights the specific temporal trajectory and dynamics for UCH-L1 Suggested effective serum half-life is noted, as derived from graphs or tables. "Notes" indicate any specific considerations or notable findings of serial sampling in the specific article. TBI, traumatic brain injury; ECLIA, electrochemiluminescent immunoassay; ELISA, enzyme-linked immunosorbent assay; GCS, Glasgow Coma Scale; ED, emergency department; GFAP, glial fibrillary acidic protein; UCH-L1, ubiquitin carboxy-terminal hydrolase L1.

peaks, or increasing trajectories in patients with serious injuries, were found in patients in a few papers (92, 111, 112, 115, 116). One study suggested that UCH-L1 peaks at around 8 h after injury (92).

Suggested Serum $t_{1/2}$ of UCH-L1

Looking at the available data, the serum $t_{1/2}$ seems to be about 10 h (12, 112, 116, 117) in severe TBI patients, a few hours shorter in milder cases (112) (**Figure 2D**). In comparison to the other markers, UCH-L1 actually had one study with the goal of establishing a "half-life" of UCH-L1, which was given at 7–9 h (115) in severe TBI patients. This was found shorter in milder TBI cohorts, where data indicated that it was around 6 h (115).

Neurofilament Light

A search for NF-L identified a total of 575 manuscripts. Following removal of duplicates and after assessing full manuscripts, only $n = 2$ articles were deemed eligible for final inclusion (Figure S4 in Supplementary Material) and are listed in **Table 5**.

Patient Characteristics

Only two studies were included which both presented NICU TBI materials with mixed TBI severity according to GCS admission (**Table 5**) (13, 56). No pediatric TBI population has been studied.

Assays to Analyze NF-L

Officially, there are currently no available ELISAs for NF-L in serum. One of the included studies instead used an ELISA assay developed for CSF samples (13), and the other used the newly developed single molecule array technique to create a functional assay (56).

Trend of NF-L over Time after Trauma

In contrast to the other serum biomarkers, the two available studies suggest that NF-L levels in serum in a mild-to-severe (and one severe) TBI cohort of NICU patients tend to increase over time during the first 1–2 weeks (increased during the whole sampled period) (13, 56). Additionally, some patients were found to have elevated levels even 1 year after trauma (56).

Suggested Serum $t_{1/2}$ of NF-L

With the data available, it is not possible to determine a serum $t_{1/2}$ for NF-L (13, 56). However, it is evident that this is the protein with the longest $t_{1/2}$ of these biomarkers.

DISCUSSION

This systematic review highlights that serial sampling of different biomarkers in serum results in distinguishably different temporal trajectories in TBI patients. Serum S100B and UCH-L1 levels seem to have the shortest $t_{1/2}$ while the serum levels of the biomarkers GFAP and NSE both remain elevated for a prolonged period of time, as compared to S100B and UCH-L1. Even more extended, NF-L appears to have the longest $t_{1/2}$ of the biomarkers. However, a specific value could not be identified in the studies, as it continued to increase over the sampling period of 2 weeks. Due to the heterogeneity of included patients, secondary brain injury development, assays used, and sampling frequency, it is impossible to draw any accurate conclusions regarding standardized elimination half-lives after concentration peaks for these proteins, but we believe our effort including effective half-lives provides the best possible attempt to date. Moreover, different sources of biomarkers seem to influence the total serum levels over time, with extracranial contribution being most influential for S100B and NSE, where this has been most extensively studied. Despite these caveats and in contrast to the other biomarkers, S100B and NSE have fully automated clinical assays, making them accessible for routine clinical use. To our knowledge, this is the first systematic review of temporal profiles of biomarkers following TBI, and it could serve as a platform to better assess and compare novel brain biomarkers to be introduced, as well as relate future studies presenting serial sampling of TBI patients.

Unsurprisingly, the searches that generated the greatest numbers of research articles were that of S100B and NSE. These markers are by far the most studied in TBI but have also been studied in other intracranial conditions, mainly in stroke (118) and to assess brain injuries in patients suffering from circulatory arrest (119). Regarding different aspects of the temporal trajectories, S100B is by far the most studied. It is becoming increasingly clear that temporal changes of S100B in serum are highly dynamic

TABLE 5 | Analysis of NF-L studies.

Reference	Number of patients	Patient characteristics	NF-L assay	Sampling frequency	Trend over time	Suggested effective half-life	Notes
Al Nimer et al. (13)	182	Adult NICU TBI patients	ELISA, Uman Diagnostics	Varying frequency first 2 weeks	Constantly increasing, unchanged over first week	N/A	No special monitoring aims
Shahim et al. (56)	72	Adult TBI patients, GCS 3–8	Simoa, Quanterix	Initially, every 24 h	Constantly increasing, group level	N/A	No special monitoring aims

Number of patients highlighted the total number of patients included in the study, sometimes highlighting in parenthesis how many were actually included with TBI or serial sampling. Patient characteristics described the age groups and injury severity level according to the GCS. The assay described the technique used for the assay and if available the manufacturer. Sampling frequency illustrates with what frequency samples were acquired. Trend over time highlights the specific temporal trajectory and dynamics for NF-L. Suggested effective serum half-life is noted, as derived from graphs or tables. "Notes" indicate any specific considerations or notable findings of serial sampling in the specific article. TBI, traumatic brain injury; NICU, neurointensive care unit; ELISA, enzyme-linked immunosorbent assay; Simoa, single molecule array; N/A, not available; NF-L, neurofilament light; GCS, Glasgow Coma Scale.

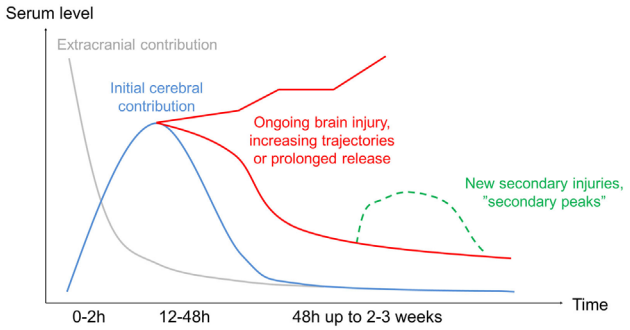

FIGURE 3 | Protein kinetics following injury. Highlighting the estimated temporal profile from trauma if frequently sampled. Initially, there will be a major release of the protein from extracranial sources (gray line), in theory more so if it is present to a larger extent in tissue likely to be injured (i.e., S100B in adipose tissue), even if this contribution generally decreases rapidly. The cerebral contribution (blue line) will continue to increase in serum (for S100B up to 27 h), presumably due to influx from the injured brain. In case of ongoing injury development, the subsequent serum samples may have a prolonged decline or even continue to increase depending on the injury severity (red line). In case of a new injury development, secondary peaks have been shown (green line). While this pattern is most studied for S100B, it applies to some extent to all the biomarkers even if the time frames are different.

following brain injury (39, 66, 73, 120). **Figure 3** is an attempt to better illustrate these changes, and while it is constructed with the dynamics of S100B in mind, it may be generally applicable to the other biomarkers as well, but with different $t_{1/2}$. The exception is NF-L, where the $t_{1/2}$ is so long that it has not yet even been estimated. The highest levels of S100B are seen early (within 60–120 min after trauma) in patients suffering from multitrauma where bone, adipose tissue, and internal organs [tissues known to express S100B as well (121, 122)] are injured (11, 48). However, these extracranial contributions of S100B will decrease rapidly, as is seen in patients with only multitrauma and without brain injury (48). Jackson et al. estimated these rapid falls of S100B to have a serum "half-life" of 198 min (first sample within 60 min) (39). Townend et al. looked at S100B in mild TBI patients and found that S100B only had an estimated "half-life" of 97 min, even though these samples were acquired a bit later after trauma (CI: 75–136 min, first sample within 240 min following trauma) and all did not have structural injuries (37). In our own experience, the highest level of S100B in serum we have seen was 23.0 μg/L (healthy reference < 0.11 and 1.0–2.0 μg/L usually seen in severe TBI patients) sampled 29 min following trauma from a patient who had fallen from the fifth floor and had severe extracranial injuries as well as intracranial focal mass lesions [patient in study (11)]. The next sample was 6.2 μg/L acquired 6.5 h after trauma, suggesting a "half-life" similar to Jackson et al. of around 3 h. It is import to realize that levels of S100B may represent two processes, where the initial early peak probably represents a more bolus-like dose of S100B assumed predominantly of extracranial origin and is eliminated quickly (**Figure 3**), here better reflecting its true serum elimination half-life. The second peak, after about 24 h, represents a slow release net sum of influx and outflux of S100B to serum, predominantly from the injured brain and where the slower decay is affected by the continued release, thus the extended $t_{1/2}$. This interpretation is supported by our study of moderate-to-severe TBI patients, where we saw that the late 24 (27.2)-h peak is highly related to outcome whereas the initial peak is not (11). We have also modeled the functional kinetics of S100B in moderate-to-severe TBI patients, after excluding this initial "trauma peak" (73). S100B was found to have an expected "brain injury" peak level at around 27 h after injury (38). After that peak, it should drop with an expected rate during the upcoming days (38). If S100B does not follow this trajectory, it could indicate ongoing brain injury (9, 43, 44), resulting in an unfavorable outcome (11). We must stress that the trajectory described here is that of TBI and has not been extensively studied in other contexts. Our experience of several thousand patients in routine clinical use, in for example patients with embolic stroke, is that they can express an extended release, often peaking at day 2–3. The cause of this is not yet understood, but could reflect ongoing penumbral leakage or patterns of recirculation. The presence of secondary peaks of S100B should be highlighted (7, 8, 44–46), as they have been shown to be associated with secondary brain injuries or neurological deterioration in TBI patients. In summary, it is important to understand the kinetic profile of S100B, and its different components when interpreting it as a biomarker of brain injury.

The second most studied protein was NSE. Similar to S100B, a steady decline is generally seen but with a serum $t_{1/2}$ longer than for S100B. However, patients with severe injuries may continue to present increasing levels in serum after trauma (47, 48). The general decrease seen for NSE may be delayed in patients with unfavorable outcome or more severe injuries (11, 44), and NSE has been shown to be influenced by extracranial contribution (11, 49), possibly more so than S100B. Another major caveat with NSE is its presence in erythrocytes making serum sampling unreliable if hemolysis is present (50) despite that there are tools attempting to adjust for this (51) and procedures in automatic clinical assays that discard. Similar to S100B, secondary peaks of NSE have been shown in patients with new or progressing injuries (44, 52). In aggregate, NSE behaves similar to that of S100B in serum, albeit it appears to have a longer contextual half-life in serum of about 48 h and has larger influence from extracranial sources.

Serial sampling of GFAP has been less commonly studied in TBI, but interest is increasing, presumably due to GFAP's superior brain specificity (41). The serum $t_{1/2}$ levels of GFAP are extended, as compared to S100B, presumably at around 24–48 h in severe TBI patients and thus similar to NSE. This prolonged increase in serum levels may prove to be beneficial for diagnostic screening of intracranial lesions in milder TBI, being more detectable >6 h after injury, as compared to S100B (53, 54). However, it appears to lack granularity to detect more rapidly changing trajectories as seen when serially sampling proteins with shorter effective half-lives, such as S100B and UCH-L1. This may explain why only a limited amount of studies report secondary peaks of GFAP (12). A long $t_{1/2}$ will make it difficult to use in assessment of treatment efficacy and monitoring, as it would in theory provide a delayed treatment response and show a blunted concentration. Despite this, a delayed decrease

or continued release of GFAP is seen in patients with unfavorable outcome (53, 55–57). Patients with mass lesions appear to have higher levels of GFAP in serum as compared to more diffuse injuries (12, 58), especially in combination with lower levels of UCH-L1 (used in a glial:neuronal ratio) (59). While GFAP has been seen to increase in patients with extracranial trauma and without brain injury (60), reports of serial sampling in multitrauma populations are scarce (108), but as the protein is so much more brain specific as compared to S100B (Table S1 in Supplementary Material; **Table 6**) (41), available data suggest that extracranial contribution over time to be relatively low. In summary, GFAP seems to have longer $t_{1/2}$ half-life than S100B, of about 24–48 h, which might prove beneficial for screening purposes if a patient is sampled late after ictus, but might decrease accuracy to detect and separate novel lesions and monitor ongoing events.

Brophy et al. analyzed serum levels of UCH-L1 with a high sampling frequency and established its serum functional half-life to be in the vicinity of 7–9 h (115). They also noted it to differ between severe and mild injury. Moreover, similar to the other markers, they discovered some individual patients with secondary increases (29, 61). This decrease was slower in patients with more severe injuries and worse outcomes (29, 53), also analogous with the other biomarkers. Interestingly, and in contrast to GFAP, diffuse injury seems to lead to higher levels of UCH-L1 as compared to focal mass lesions (12). UCH-L1 is more brain specific than S100B (41), but data indicate that it is also significantly increased in patients with extracranial injuries (60). In aggregate, UCH-L1 appears to have a relatively short functional half-life, similar to that of S100B, but needs further studies to elucidate its temporal profile following trauma as well as more robust associations with extracranial injuries.

The protein NF-L is the least studied in a temporal context, presumably because no commercial assay is available at present. The two studies investigating this biomarker in TBI populations noted that serum levels of NF-L continually increased the first week(s) after injury (13, 34). There are no *in vivo* studies that have appropriately assessed the serum half-life of NF-L, but an *in vitro* report suggests that it may be as long as 3 weeks (62), which could be possible looking at available data. Surprisingly, it was found elevated even at up to a year in some patients (34), perhaps indicating ongoing pathology. Neurofilament heavy (NF-H) is another, similar, axonal protein that has been studied in TBI and shows similar trends with continually increasing serum levels the first days after trauma (63). A case series suggests that NF-L may aid in assessment of diffuse axonal injury (64), and a study indicates that it adds independent information in outcome prediction models, in addition to S100B (13). As reliable assays become more readily available, there might be a growing interest in this marker, which could reflect an ongoing neuroinflammatory pathology, distinctly different to the others studied here. However, considering its serum dynamics, it would probably provide little information on the rapid development of novel intracranial lesions the first week in the NICU but would instead be of greater interest in later, more chronic phases of TBI.

TABLE 6 | Characteristics of the selected protein biomarkers.

Protein	Molecular weight	Primary origin	Automated assay	Extracranial contribution	Effective serum half-life	Clinical relevance
S100B	9–11 kDa	Astrocytes	Available	Relatively high	Short (hours up to 24 h)	+ Effective for serial sampling and monitoring purposes, can detect secondary deterioration. + Well validated in the literature. – Extracranial contribution lowers its potential early after multitrauma.
Neuron-specific enolase	47 kDa	Neurons	Available	Relatively high	Long (24 h–3 days)	+ Rather well validated in the literature, have been shown to detect secondary deterioration. – Hemolysis leads to high levels in serum. – Extracranial contribution lowers its potential in multitrauma. – Relatively long effective half-life limits the potential for monitoring.
Glial fibrillary acidic protein	50 kDa	Astrocytes	Not available	Very low	Long (24 h–2 days)	+ Low extracranial contribution. + Rather well validated in the literature, have been shown to detect secondary deterioration. – Relatively long effective half-life limits the potential for monitoring.
Ubiquitin carboxy-terminal hydrolase L1	25 kDa	Neurons	Not available	Low	Short (hours up to 12–24 h)	+ Low extracranial contribution. + Should be effective for serial sampling and monitoring purposes because of short effective half-life. – Limited validation in the literature, but has been shown to detect secondary deterioration.
Neurofilament light	68–70 kDa	Neurons	Not available	Very low	Very long (3 weeks?)	+ Low extracranial contribution. – Very long effective half-life limits the potential for monitoring. – Limited validation in the literature.

Illustration of some of the protein characteristics. Primary origin indicates which cell in the central nervous system contain highest amount of the specific protein. Molecular weight is the size of the protein in kilo Dalton and the primary cellular origin is the cells with highest amount of expressed concentration in the central nervous system. If an automated clinical assay platform is available, it is indicated. Extracranial contribution is an aggregate from Table S1 in Supplementary Material, indicating how much protein and mRNA that is expressed outside the central nervous system. Serum effective half-life is an aggregate of the findings in this study. Clinical relevance is exemplified.

In **Table 6**, we present an aggregate of our findings on these markers. Our review finds the effective serum half-lives of these biomarkers to be similar in ranges to those suggested by a recently published narrative review of the field where, among others, S100B, NSE, GFAP, and UCH-L1 were included (65). In contrast to that narrative review, we have focused on the serum compartment, attempting to systematically interpret information on effective half-lives from all available studies. While it was impossible to conduct a proper meta-analysis, we summarized the available literature in histograms for S100B (**Figure 2A**), NSE (**Figure 2B**), GFAP (**Figure 2C**), and UCH-L1 (**Figure 2D**), where it is possible to see that S100B and UCH-L1 have the most amount of studies that indicate a shorter effective serum half-life while GFAP and NSE exhibit relatively longer serum $t_{1/2}$ (**Figure 4**).

Many factors could affect the contextual half-lives of the studied biomarkers, and we acknowledge that the serum effective half-lives provided here might still be inaccurate. While we excluded studies without structural imaging, we did not look at lesion progression as only one study properly reported this (9), something we believe will influence trajectories of serially sampled proteins. The association between lesion size and biomarker levels was also barely mentioned (66). Together, these conditions make it difficult to accurately generate a precise half-life due to the constant influx/efflux of the proteins to the serum compartment (20). Furthermore, some studies suggest peak times of biomarkers as related to time after trauma (73, 102, 112, 115). Currently, S100B is the only protein where attempts have been made to model the temporal contextual kinetics following injury (73), something that also needs to be performed for the others. We believe that all of these serum proteins, except for NF-L, peak relatively early in serum and any unexpected prolonged release might not be a natural progression but could indicate deterioration or ongoing damage in some way. Moreover, it is unknown to what extent these proteins are expressed after trauma, also potentially affecting levels. Additionally, extracranial injuries give rise to some of these proteins in serum as well, primarily NSE and S100B, resulting in altered serum dynamics (11, 48). While UCH-L1 and GFAP have been shown to be increased in serum of non-head injured patients as well (123), they are, together with NF-L, more brain specific (122). We show this in Table S1 in Supplementary Material, where we have gathered protein and mRNA expression (in tissues usually injured in multitrauma patients). In aggregate, extracranial contribution and injury progression need to be better assessed in future studies as it will affect contextual kinetics.

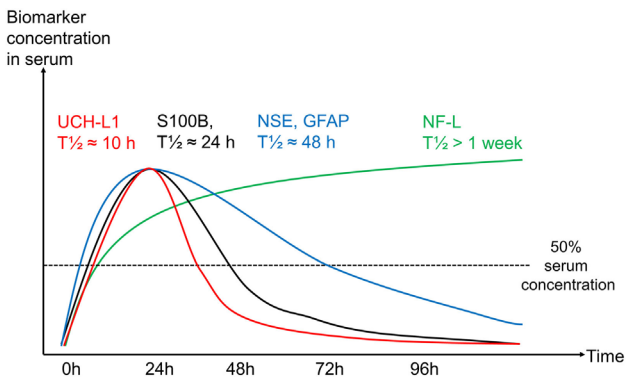

FIGURE 4 | Protein kinetics for each protein in serum over time. Graph illustrating how the influence of an increasing effective half-life results in a serum sample in an uneventful traumatic brain injury (TBI) (without secondary deterioration) for ubiquitin carboxy-terminal hydrolase L1 (UCH-L1) (red), S100B (black), neuron-specific enolase (NSE)/glial fibrillary acidic protein (GFAP) (blue), and neurofilament light (NF-L) (green). Biomarker concentration in serum on *y*-axis and time in hours on *x*-axis. Note that these are estimates based on the knowledge of S100B kinetics in serum, current literature makes it difficult to illustrate more accurate trajectories over time for the other proteins.

Several articles mentioned refer to the term "serum half-life" when trying to describe the temporal profiles following TBI. We believe that this description is inaccurate as we are not looking at protein decay in a single space; instead it is probably a combination of influx and efflux between bodily compartments with ongoing release from the injured brain, where actual clearance is one of many actors (20). Thus, we have used the term "effective serum half-life" to describe that concentration dynamics is presumably more accurate as this is not a process with constant decay (i.e., as is true in theory for a biological half-life).

We could not find any signs that younger patients should have a different temporal profile than adults for these proteins. Instead, and as can be seen in the tables, biomarker dynamics in serum appear to be correlated with injury severity. However and notably, pediatric populations were not nearly as frequently studied as adult TBI patients. The reference levels for healthy pediatric populations (especially the first year) of S100B and NSE have both been shown to be significantly higher than for adults (factor ×2–4) (124, 125). Nevertheless, during traumatic conditions, similar trajectories to adults are seen in pediatric populations, and serum dynamics are likely a marker of injury severity and progression not requiring separate reference levels per age group. In aggregate, age does not seem to play a major role in biomarker serum dynamics, but is not as well studied in pediatric populations as compared to adults.

The literature varies greatly in terms of the sampling frequency chosen. This may be problematic when attempting to determine the detailed kinetics. For many studies, the generally long sampling intervals chosen severely limit our knowledge of its early behavior. The choice of optimal sampling frequency to ensure faithful replication of a time series has received extensive investigation in information theory (126). In essence, the sampling frequency must be chosen to be at least twice the characteristic frequency of the signal. In other words, if changes are expected over a particular time period, then the sampling interval must be at most half of this and preferably more frequent still. Thus, we suggest that future prospective studies consider the following issues:

– Sampling frequency: It is best advised to perform a high initial sampling frequency to accurately map trajectories over time (proteins like S100B/UCH-L1 needs a higher frequency than NF-L). If early detection is the goal, then a tapered strategy may help identify peak with early frequent sampling.

- Relations to imaging: A high frequency of imaging will best aid association of biomarker trajectories with potential injury progression. Current imaging modalities in practice limit the frequency possible.
- Relations to other monitoring: High frequency multimodal monitoring (metabolism, oxygenation, intracranial pressure, etc.) may help associate biomarker trajectories with potential secondary insults/deterioration.
- TBI population: We suggest to identifying and studying TBI cohorts that are clinically and pragmatically definable such as NICU TBI patients, thus aiming to understand a biomarker in the context of the population it is expected to be clinically used in.
- Blinding: Serum biomarkers should be analyzed in retrospect or blinded as to not influence treatment strategies in a study setting.
- Analysis method: If possible, use a well validated assay, preferably with industrial-level calibration.

Readily available and reliable assays are crucial if protein biomarkers are to find routine clinical application. To date, automated assays with industrial calibrations are only available for S100B and NSE, and this makes it possible to provide reliable analyses in less than 20 min from sampling. Until this is widespread, it will be difficult for the other proteins to reach everyday use as these assays (i.e., ELISAs) take around 6 h or more to run. Moreover, without proper automatic assays with regular, standardized calibrations, there is a risk for greater inter- and intravariability between samples and studies, as has been seen between ELISA methods for S100B (67). Actually, we noticed that several studies that did use ELISA for NSE and S100B showed a different release pattern after trauma with consisting higher levels over time, as compared to the automated assays, resulting in longer serum half-lives (66, 68, 69). This is worrying as it may imply that the assays (and thus the studies) are not as comparable as has been suggested, presumably due to different antibodies used or different lower levels of detection, stressing the need for standardized testing in the field. Likely, while we did not particularly focus on the exact levels but on the temporal profiles, they would also be affected by this.

Limitations

We aimed to perform a meta-analysis of the data collected but realized that this was not possible primarily due to the use of different assays, differences in sampling time and heterogeneous patient populations. Instead, we have listed estimates of serum half-lives by assessing graphs, tables, and data sets from previous studies, which we believe generates the best possible current estimates of the effective serum half-lives of these proteins after TBI.

Studies of S100B and NSE are more frequent than studies analyzing UCH-L1 and NF-L. Results concerning the proteins with little data should be interpreted with more caution. In the case of NF-L, only two studies were available and the uncertainty is large here. Indicative of this is that in contrast to the findings on NF-L, NF-H has been found to have a short half-life in mild TBI $t_{1/2}$ (48–72 h) (127). As these two components may have similar half-lives and as no mild TBI study is available for NF-L, it is possible that NF-L has a shorter $t_{1/2}$ in this population as well.

It is possible that several papers coming from the same research centers contain, to some extent, the same patients several times. We have not been able to adjust for this possibility. We mention all studies in the tables but focus on the largest patient cohort from each group in the Section "Discussion."

While we have acknowledged a difference in sampling frequency between basically all studies, one further issue is that a majority of studies report sampling since admission, not from the actual trauma time. As the dynamics for a protein such as S100B differs substantially in time the first 24 h (38), having the exact trauma time reported is essential to generate adequate models of biomarker release.

CONCLUSION

It is increasingly apparent that the dynamic behavior of serum TBI biomarkers varies greatly and an appreciation of this is critical for their interpretation as markers of tissue fate. The initial intracranial injury, potential extracranial trauma as well as injury progression and the occurrence of secondary injuries will influence the biomarker temporal profile. Unfortunately, while serial sampling is common in studies, few adequately comment on the temporal profiles of the analyzed proteins and even less address what sampling frequency is needed to capture information content. From a clinical perspective, and with the aim of using biomarkers as ongoing monitors of TBI patients, proteins with shorter serum availability such as S100B and UCH-L1 may be advisable as compared to proteins such as NSE, GFAP, and NF-L, as the longer peak times and half-times may lag detection of secondary harmful events. Moreover, brain specificity of the proteins should be taken into account and the need for fast, reliable assays is the definite current rate-limiting step in research that may lead biomarkers to clinical use. Further prospective research on the contextual kinetics of protein biomarkers is urgently warranted if their full diagnostic potential is to be realized.

AUTHOR CONTRIBUTIONS

ET, DN, AE, FZ, AB, B-MB, AH, SM, and DM designed and planned the study; drafted the manuscript which all authors read and approved. ET conducted the systematic review with the help from FZ.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fneur.2017.00300/full#supplementary-material.

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Current Opportunities for Clinical Monitoring of Axonal Pathology in Traumatic Brain Injury

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Traumatic brain injury (TBI) is a multidimensional and highly complex disease commonly resulting in widespread injury to axons, due to rapid inertial acceleration/deceleration forces transmitted to the brain during impact. Axonal injury leads to brain network dysfunction, significantly contributing to cognitive and functional impairments frequently observed in TBI survivors. Diffuse axonal injury (DAI) is a clinical entity suggested by impaired level of consciousness and coma on clinical examination and characterized by widespread injury to the hemispheric white matter tracts, the corpus callosum and the brain stem. The clinical course of DAI is commonly unpredictable and it remains a challenging entity with limited therapeutic options, to date. Although axonal integrity may be disrupted at impact, the majority of axonal pathology evolves over time, resulting from delayed activation of complex intracellular biochemical cascades. Activation of these secondary biochemical pathways may lead to axonal transection, named secondary axotomy, and be responsible for the clinical decline of DAI patients. Advances in the neurocritical care of TBI patients have been achieved by refinements in multimodality monitoring for prevention and early detection of secondary injury factors, which can be applied also to DAI. There is an emerging role for biomarkers in blood, cerebrospinal fluid, and interstitial fluid using microdialysis in the evaluation of axonal injury in TBI. These biomarker studies have assessed various axonal and neuroglial markers as well as inflammatory mediators, such as cytokines and chemokines. Moreover, modern neuroimaging can detect subtle or overt DAI/white matter changes in diffuse TBI patients across all injury severities using magnetic resonance spectroscopy, diffusion tensor imaging, and positron emission tomography. Importantly, serial neuroimaging studies provide evidence for evolving axonal injury. Since axonal injury may be a key risk factor for neurodegeneration and dementias at long-term following TBI, the secondary injury processes may require prolonged monitoring. The aim of the present review is to summarize the clinical short- and long-term monitoring possibilities of axonal injury in TBI. Increased knowledge of the underlying pathophysiology achieved by advanced clinical monitoring raises hope for the development of novel treatment strategies for axonal injury in TBI.

Keywords: traumatic brain injury, diffuse axonal injury, monitoring, neurocritical care, neuroimaging, biomarkers, microdialysis

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INTRODUCTION

Traumatic brain injury (TBI) is a significant cause of morbidity and mortality worldwide (1–5). Mortality due to severe TBI can reach 40% with high rates of disability among the survivors (6–8). Cognitive, behavioral, and emotional impairments are common and particularly disabling post-TBI and can persist into the chronic stage (9–12). Widespread injury to the white matter tracts, a key feature of TBI, disrupts neuronal networks and impairs information processing which contributes to the cognitive impairments observed post-TBI (9–11).

Axonal injury was initially described by Strich in 1956, who observed diffuse axonal degeneration at autopsy of severe TBI patients (13). Axonal pathology was later established as a separate TBI entity by Adams and colleagues in 1982 (14). When axonal damage occurs in multiple brain locations in clinical TBI, it is named diffuse axonal injury (DAI) (15–21).

In the preclinical setting, the term traumatic axonal injury has been applied to describe axonal damage, with the term DAI used to express its clinical counterpart (5, 19). Thus, DAI is a clinical entity characterized by radiological and/or histological findings suggestive of axonal pathology at certain predilection sites, particularly the gray/white matter interface, the corpus callosum, and the brain stem (14, 15). DAI remains a challenging clinical entity with a frequently unpredictable course and outcome. To date, there are limited therapeutic options reflecting the incomplete knowledge of the underlying pathophysiology of DAI. However, it has been established that axonal injury results from a highly dynamic process involving a cascade of events that may evolve over time leading to progressive white matter atrophy with variable clinical impact (19, 22–24). Therefore, detection and monitoring of DAI is relevant from the acute to chronic phase, in order to evaluate its severity, seek treatment options and better predict clinical outcome.

In this review, an overview of the current clinical possibilities for monitoring of DAI is provided. A literature search was performed in PubMed, Scopus and ISI Web of Knowledge. Experimental/preclinical studies on axonal injury in TBI were excluded from the overview with the exception of those describing key pathophysiological mechanisms. Articles where the injury type was not mentioned, was unclear or encompassing only focal TBI were also excluded from the analysis. Articles on mild, moderate, and/or severe TBI, DAI, and traumatic axonal injury were extracted, further screened and were included if the investigated mechanisms involved aspects of axonal/white matter injury. The literature on mild TBI, considered a diffuse TBI subtype with features of axonal and white matter pathology, was thus also included in our search.

BIOMECHANICS AND STRUCTURAL CHANGES IN DAI

The predominant mechanism in the development of axonal injury in TBI is mechanical shearing and stretch forces produced by inertial acceleration/deceleration stresses to the head (25–28) (**Figure 1**). This inertial loading triggers dynamic shear, tensile, and compressive strains within the brain. Consequently, certain parts of the brain move at a slower pace relative to others, leading to deformation of the brain tissue (17, 29). In the preclinical TBI setting, unmyelinated axons sustained more injury compared to myelinated ones, suggesting that axons are unequally vulnerable (30).

Under normal conditions, brain tissue can withstand stretches and easily return to its original geometry without any resulting injury. In contrast, when the strain is rapidly applied, the brain tissue loses its plasticity and acts stiffer, becoming more vulnerable and brittle (31). In particular, axons in the white matter appear poorly prepared to withstand injury from rapid mechanical brain deformation at time of TBI (17), resulting in injury to the axonal cytoskeleton (17, 32, 33). Nevertheless, the development and severity of axonal injury is dependent on both the magnitude and rate of strain during impact (17).

Primary axotomy in humans is rare with secondary (delayed) axotomy being the most likely mechanism leading to axonal disconnection (5, 19, 34, 35). Experimental evidence indicates that mitochondrial dysfunction (5, 36), as well as TBI-induced inflammatory responses, contribute to the secondary axonal injury (5, 37, 38). In addition, impaired axonal transport causes axonal swelling over time post-injury, leading to the accumulation of numerous potential biomarkers which may then be released into the surrounding tissue (see later section of this review).

CLINICAL FEATURES AND NEUROCRITICAL CARE MONITORING OF AXONAL INJURY

Clinical Characteristics

From a clinical point of view, initial loss of consciousness and coma as well as later features such as prolonged vegetative state or cognitive impairment can be characteristics of both focal TBI and DAI, although may be more frequently observed in the latter (29, 39). The presence of a decreased level of consciousness and coma is commonly a result of axonal injury in the diencephalon and/or the brain stem (29, 40, 41). In DAI survivors, cognitive dysfunction, mood disorders, and behavioral problems are frequent and result in a decreased quality of life (17, 29, 42). In particular, memory impairment and problems in executive functioning are frequent in DAI (43, 44), and so is impaired information–processing speed (45). In addition, motor weakness may be caused by injury to the pyramidal tract (46), which is more frequently encountered in DAI compared to focal TBI (29).

By definition, axonal injury is difficult to diagnose using only clinical signs and symptoms. Some clinical features of axonal injury are presumably to a large extent related to the anatomic distribution of DAI. Based on the limited ability of computed tomography (CT) and standard T1- and T2-weighted magnetic resonance imaging (MRI) sequences to precisely detect the underlying axonal injury in TBI, it has been difficult to confirm and diagnose DAI with certainty (17). However, neuropsychology testing after release from hospital can detect cognitive and memory deficits as well as

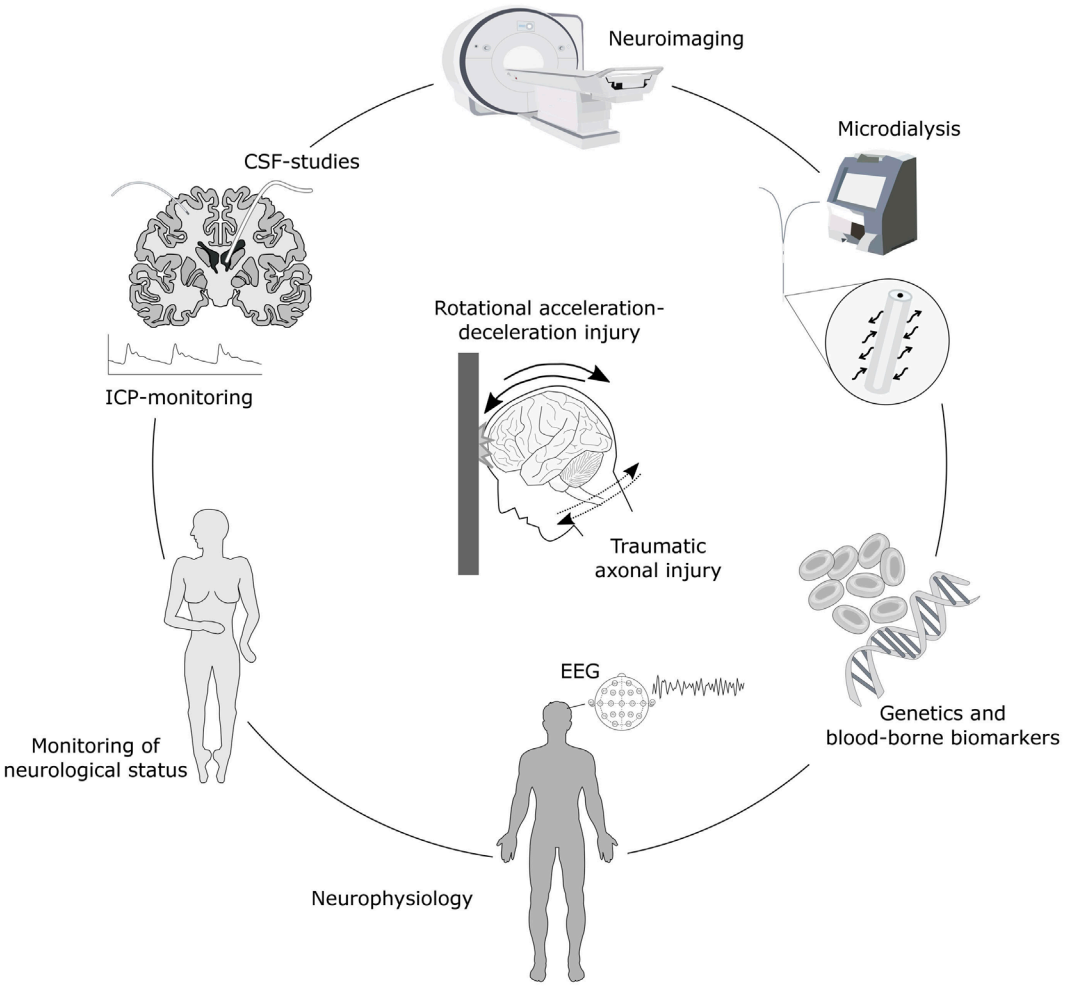

FIGURE 1 | Schematic illustration of monitoring options for axonal injury. Biomechanically, traumatic axonal injury results from head impact with rotational acceleration-deceleration forces. Detection and monitoring of axonal injury is possible with numerous advanced neuroimaging techniques such as magnetic resonance imaging (MRI), including diffusion tensor imaging (DTI) and magnetic resonance spectrometry (MRS), as well as neuromolecular imaging by single-photon emission computed tomography (SPECT) and/or positron emission tomography (PET). Axonal injury also results in the secretion of various biomarkers into the interstitial fluid (ISF), cerebrospinal fluid (CSF) and the bloodstream which can be detected in ISF using microdialysis, in CSF by sampling through an external ventricular drainage or through lumbar puncture, and in serum by blood sampling. These biomarkers provide clues of temporal patterns of axonal injury and ongoing secondary injury processes and may be associated with outcome. Monitoring of axonal injury progression may also be achieved by placement of an intracranial pressure (ICP) monitoring device for continuous surveillance of ICP, neurophysiological methods such as electroencephalography (EEG) and periodic assessments of neurological status including level of consciousness. Furthermore, the genetic profile may add additional information of risk for secondary injury cascades and neurodegenerative development.

slowed mental processing, characteristics which, when combined with knowledge of the underlying injury mechanisms (**Figure 1**) and neuroimaging findings, may be highly suggestive of DAI. These features can help confirm the diagnosis of moderate or severe DAI with a relatively high degree of certainty in TBI patients (29, 47, 48).

In addition, advanced neuroimaging has recently enabled improved visualization of surrogate markers for the histopathological features of DAI, and for subsequent surveillance of secondary injury processes. Injury to white matter tracts interconnecting cortical regions, disrupting large scale brain networks of particular importance for complex cognitive functions (49, 50), are now possible to estimate using modalities like diffusion tensor imaging (DTI) and be correlated with cognitive and behavioral deficits observed using neuropsychology testing (10, 50, 51).

Intracranial Pressure (ICP) Monitoring of DAI

Intracranial pressure monitoring remains a cornerstone in the management of severe TBI patients, although the incidence of raised ICP in DAI is not well established. Maximum ICP has been correlated to the number of identifiable white matter lesions on MRI (52), and a relationship with the Marshall CT classification score and ICP levels was suggested (53, 54). In some studies of severe DAI patients, ICP was not elevated (55), whereas others found increased ICP in most TBI patients (56–58). In an early

study of ICP monitoring in TBI patients, of whom 61 had a normal CT scan, no ICP elevations were observed unless the patient was aged >40 years, had unilateral or bilateral motor posturing or episodes of systolic blood pressure <90 mmHg (59). In contrast, another study found elevated ICP despite the absence of mass lesions, midline shift, or compressed basal cisterns on the initial CT scan (60). Later, less ICP elevations were observed in DAI compared to other TBI subtypes and it was suggested that ICP monitoring could be omitted in DAI (55). However, in that particular study, 10% of patients had ICP >20 mmHg, and two patients required treatment for elevated ICP. Similar patterns of transient ICP elevations triggered by neurocritical care events in DAI patients were also observed (61). Recently, ICP was analyzed in MRI-verified DAI patients, and although persistently raised ICP during the first 96 hours of monitoring was not seen, 20% of patients required treatment for transient ICP elevations (62).

Thus, the use of ICP monitoring in DAI is controversial (63). Although it was suggested that individuals with DAI documented by neuroimaging may not require treatment for elevated ICP, high ICP values were still frequently encountered in such patients. The use of ICP monitoring in comatose patients with initial normal CT scan or CT scan with minimal findings has also been questioned and recommended only in the presence of radiological worsening (64). On the other hand, in comatose patients with diffuse TBI with evidence of brain swelling on CT scan, ICP monitoring is indicated in the early post-injury period (64). It should be also noted that DAI patients with effaced basal cisterns on CT scan carry a high risk of increased ICP (58, 65).

In summary, studies evaluating the incidence of elevated ICP in DAI patients are scarce and provide contradictory results. Repeated clinical examinations and neuroimaging may be possible alternatives for monitoring of DAI patients when the initial CT scan is free from or shows only minimal abnormalities, since these patients may have a low risk of intracranial hypertension (63). Nevertheless, although it has not been firmly shown that outcome is improved, ICP monitoring in DAI patients with reduced level of consciousness and pathological findings on CT scan is recommended in the initial post-injury period (64, 66, 67).

Monitoring of Cerebral Blood Flow (CBF) and Brain Oxygenation

Perfusion CT or xenon-enhanced CT (Xe-CT) are both rapid and widely available techniques for the evaluation of CBF. For Xe-CT, a mobile CT scanner enabling bedside measurement of CBF is used (68). Although clinical experience in DAI is still limited, significant CBF alterations seem less frequent than in focal TBI (69–71). These imaging techniques, however, allow only intermittent CBF measurements and transient CBF impairment in the intervals between examinations cannot be established. Continuous monitoring of CBF is possible using thermal diffusion or laser Doppler methods, both requiring insertion of an intraparenchymal probe to assess focal CBF in a small brain volume (72, 73). Clinical experience with these techniques is still limited, and to date, there are no studies specifically evaluating local CBF measurements in DAI.

Cerebral blood flow may also be indirectly estimated using jugular venous oxygen saturation ($Sjvo_2$) and brain tissue oxygenation ($PBto_2$). $Sjvo_2$ can be measured using a fiberoptic probe placed in the jugular bulb and ranges between 55–75% under normal conditions. Low $Sjvo_2$ values may suggest hypoperfusion and ischemia and episodes of desaturation correlate with poor outcome (74). On other hand, high values >75% may represent hyperemia and also correlate with brain infarctions, since oxygen is not extracted from irreversibly injured brain tissue.

$PBto_2$ measurements require a sensor to be inserted in deep white matter, and allow regional measurements of cerebral oxygenation. In the uninjured brain, $PBto_2$ values are >20 mmHg while critical hypoxia may develop with values <10 mmHg. Although reductions of $PBto_2$ have been associated with poor outcome in TBI (75), and current treatment recommendations suggest interventions when $PBto_2$ falls below 15 mmHg (76), no studies have to our knowledge focused on the clinical impact of $PBto_2$ in DAI patients.

Available methods for CBF measurements as well as brain oxygenation cannot be firmly recommended in DAI in view of the limited clinical experience with these methods. Nonetheless, they are expected to play a greater role in the future especially in multifocal/mixed cases with elevated ICP and impaired CPP as a complement to ICP-CPP guided treatment protocols.

Electroencephalography (EEG)

In TBI patients, continuous EEG (cEEG) has been proven useful for the monitoring of seizure activity and the depth of sedation especially in those on barbiturate coma (77, 78). The use of cEEG in TBI is also indicated for the detection and treatment of non-convulsive seizures (NCS), a common risk in severe TBI patients (79, 80). Although only low quality evidence exists, cEEG monitoring may be recommended in TBI patients with unexplained behavioral alterations or sudden changes in mental state and/or altered consciousness, and to rule out NCS especially in penetrating injuries, large intracranial lesions, and depressed skull fractures (79).

There is limited data on the use of EEG/cEEG in the monitoring of DAI. In a study of 90 patients after diffuse TBI, where EEG recording was applied in the early post-injury phase, the EEG patterns correlated with prognosis (81). Specifically, most DAI patients with "benign" EEG patterns (stage 1; normal records with preserved activity, stage 2; reactive with rhythmic theta activity dominant, stage 3; usually reactive spindle coma where sleep patterns of stage 2 demonstrated rhythmic spindles) survived while most patients with "malignant" EEG findings (low amplitude delta activity, burst suppression pattern, alpha pattern coma) died (81). Following blast TBI, typically resulting in a degree of white matter/axonal injury, reduced EEG phase synchrony in the frontal area was associated with axonal injury on DTI (82, 83).

To date, the role of EEG in the monitoring of DAI has not been established. Although there is evidence to support that cEEG monitoring may be useful for the diagnosis of NCS in severe TBI, there is insufficient data in DAI at present. This monitoring modality has also not been shown to improve outcome and/or alter treatment in DAI patients. To date, it should primarily be regarded as a scientific tool awaiting additional studies evaluating its clinical role in the multimodality monitoring of DAI.

NEUROIMAGING

In recent years, advances in neuroimaging have facilitated DAI diagnostics as well as allowed for more accurate prognosis and monitoring of ongoing, secondary axonal injury. While image acquisition speed, accessibility and accuracy in detecting traumatic intracranial hemorrhages make CT the leading neuroimaging modality in the acute evaluation of TBI patients, its utility in DAI is limited. Although traumatic edema of the brain and petechial hemorrhages in the white matter indicate DAI, CT is generally insensitive for subtle axonal lesions. Hemorrhagic lesions in deep-seated predilection sites for DAI such as the corpus callosum and the rostral brain stem are rarely seen on CT. In addition, non-hemorrhagic lesions, which have been linked to poor outcome (84, 85), are not detectable (86). As discussed previously, there is a degree of axonal injury caused by the initial impact although most axonal pathology in DAI is a delayed secondary event evolving over days to weeks resulting in clinical deterioration of the patient. Thus, the admission CT in combination with the clinical picture following TBI may indicate DAI, but for the confirmation of diagnosis, monitoring of the progression of axonal injury and adequate prognosis, more advanced imaging modalities are needed.

Magnetic Resonance Imaging

Magnetic resonance imaging is a more sensitive modality for visualization of DAI-associated lesions and can detect microscopic amounts of blood, as well as non-hemorrhagic lesions secondary to axonal strain (**Figure 2**). In addition, MRI provides means to assess and visually reconstruct white matter tracts following DAI, with high sensitivity for lesion detection using DTI. Furthermore, neurochemical alterations following axonal injury can be detected with magnetic resonance spectrometry (MRS).

Conventional MRI Sequences for DAI Monitoring

Magnetic resonance imaging sequences sensitive to hemorrhagic lesions include T2*-weighted gradient echo (T2*GRE)

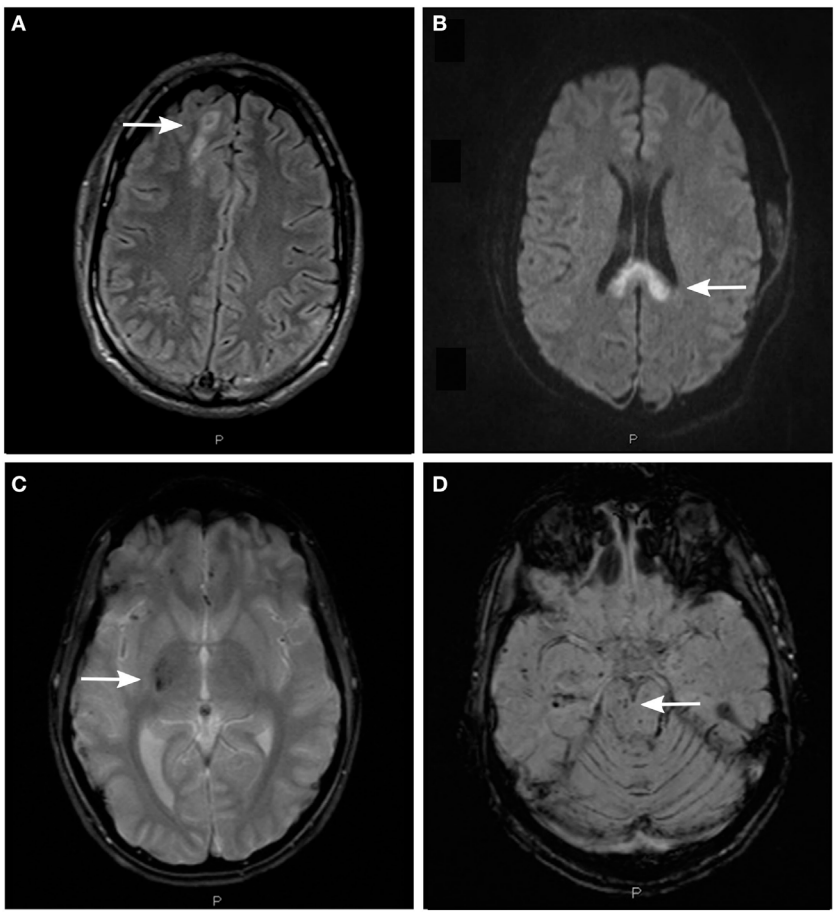

FIGURE 2 | Detection of axonal injury with conventional magnetic resonance imaging (MRI) using different MRI sequences. **(A)** Fluid-attenuated inversion recovery (FLAIR) image depicting non-hemorrhagic diffuse axonal injury (DAI)-associated lesions in the subcortical white matter of the right cerebral hemisphere (arrow). **(B)** Diffusion-weighted image (DWI) depicting non-hemorrhagic DAI-associated lesions in the body and splenium of the corpus callosum. **(C)** T2*-weighted gradient echo (T2*GRE) image depicting hemorrhagic DAI-associated lesions in the right thalamus and putamen (arrow). **(D)** Susceptibility-weighted image (SWI) depicting hemorrhagic DAI-associated lesions in the right mesencephalon (arrow) and in the white matter of right temporal lobe.

and susceptibility-weighted imaging (SWI). Both sequences can detect microhemorrhages, taking advantage of the paramagnetic properties of hemoglobin degradation products. The lesions are typically seen as small hypointense foci in DAI predilection sites and appear larger than their true size due to the magnetic field distortion. Hemorrhagic lesions seem to be fairly stable over time although some reduction of lesion numbers may be seen in the chronic phase following DAI (84, 87). Adding sensitivity of microhemorrhage detection in deep-seated brain regions, SWI has emerged as a preferred MRI sequence (88, 89) particularly in brain regions such as central brain stem previously difficult to assess (62). However, this sequence may be more complicated to interpret, since deoxygenated blood in veins can mimic hemorrhagic lesions. Nonetheless, lesions seen on SWI sequence correlate strongly to outcome (62, 90), in contrast to T2*GRE (62, 87, 91).

The Fluid-Attenuated Inversion Recovery (FLAIR) sequence facilitates detection of non-hemorrhagic lesions adjacent to cerebrospinal fluid (CSF) spaces. This sequence is useful for visualizing axonal injuries in periventricular white matter, the corpus callosum and the brain stem (84, 85). However, its capacity to visualize axonal injury is highly dependent on the timing. Lesions demonstrated in the acute phase represent tissue edema, but some seem to disappear already by 3 months post-injury (84). On the other hand, FLAIR lesions represent encephalomalacia (softening or loss of brain tissue) or tissue gliosis at the long-term, chronic phase in DAI (92).

The diffusion-weighted imaging (DWI) sequence is sensitive to the microscopic motion of water molecules (93), allowing for excellent detection of non-hemorrhagic lesions following axonal shearing. Lesions on DWI seem to correlate with initial glasgow coma scale (GCS) score and coma duration in DAI (91), and are associated with poor outcome in pediatric TBI (94). Specifically, the DWI lesion load in the corpus callosum may be of particular importance (85). However, similar to the FLAIR sequence, timing of the MRI scan is imperative when assessing DWI images (95) with the number of lesions being significantly reduced at 3 months post-TBI (84).

MRI can be difficult to obtain, particularly in the critically ill, since patients' transfer to the MRI facility may be prevented by clinical instability from intracranial and/or systemic causes. In situations where the time for MRI must for clinical reasons be extended beyond the acute phase, hemorrhagic lesions depicted in particular on SWI sequence seem to have higher prognostic value than other MR sequences. It is notable that conventional MRI sequences may still be insensitive to microstructural damage to axons and injury to white matter tracts of clinical significance can still be missed with this imaging modality (96).

Diffusion Tensor Imaging

By using image acquisition in multiple directions, the anisotropic diffusion of water molecules can be used to create DTI, providing anatomical reconstruction images of white matter tracts and quantitative measurements of axonal injury (97). DTI is more sensitive for DAI than conventional MRI, and can be used to visualize ultrastructural changes. By adding post-processing techniques to the DTI data, diffusion tensor tractography can visualize the three-dimensional anatomy of white matter tracts (98, 99). Reduction of fractional anisotropy (FA) and increased diffusivity are observed following DAI in numerous studies (100–102), they correlate to TBI severity (103, 104) and are strongly associated to cognitive and behavioral deficits in both adult and pediatric patients (10, 105–110). In addition, DTI detection of axonal injury has been cross-validated using microdialysis (MD), where FA reduction correlated to interstitial fluid (ISF) tau levels (111). As for monitoring of axonal integrity, DTI parameters have shown signs of ongoing microstructural changes long after the acute phase (105, 107, 112–114). Longitudinal studies suggest continuous changes of DTI parameters, where FA decreases over time while diffusivity increases following DAI (102, 112–114). Measurable deterioration of white matter integrity continues beyond 24 months post-injury (105, 112–115), but may stabilize thereafter (105, 115).

In summary, DTI is a robust tool to visualize posttraumatic white matter abnormalities. However, variations in data acquisition, analysis techniques, spatial location of investigated structures, lack of correlation with clinical findings, and costs (116) still impede generalized conclusions of its applied utility in DAI. Moreover, DAI lesions seen on DTI are currently used predominantly for diagnostic purposes since patient management remains predominantly symptomatic awaiting the implementation of novel pharmacological treatments.

Magnetic Resonance Spectroscopy

Magnetic resonance spectroscopy takes advantage of the chemical shift, a phenomenon caused by variations of proton resonance due to the local chemical environment. Although MRS has a low spatial resolution in comparison to MRI, it provides a mean of detecting and quantifying neurochemical alterations (117). *N*-acetyl aspartate (NAA), a marker for neuronal and axonal integrity found in high concentrations in neurons (118), and choline (Cho), which is increased after damage to cell membranes (119), are well-studied metabolites in relation to TBI. In most studies, decreased NAA and increased Cho is observed and in mild TBI, the NAA and Cho levels can appreciate axonal injury undetected by conventional MRI (120–124). These MRS findings are associated with neurocognitive deficits (125–127) and global outcomes (120, 121, 128). Longitudinal studies suggest recovery of decreased NAA levels in patients with mild DAI and/or better outcomes, implying marginal dysfunction of neurons and restoration of function over time (120, 122). However, complete recovery of NAA levels may also be possible following severe DAI (129).

Thus, MRS provides a mean for detecting and monitoring alterations in brain chemistry following DAI, although its clinical utility in DAI patients has still not been well defined. This is likely due to the lack of standardized protocols for measurements and interpretation of metabolite concentrations, a shortcoming that needs to be addressed in future studies.

Neuromolecular Imaging

Single-Photon Emission Computed Tomography

Single-photon emission computed tomography (SPECT) uses radiopharmaceutical agents to produce images of physiologic or pathological processes. For TBI, [^{99m}Tc] Hexamethylpropyleneamine oxime (HMPAO) and [^{99m}Tc] Ethylcisteinate dimer are the most widely used agents for evaluating regional cerebral

blood flow (rCBF) and indirectly, regional cerebral metabolism (130). Following DAI, decreased rCBF commonly involving the cingulate gyrus revealed signs of frontal lobe dysfunction, despite the absence of distinct anatomical abnormalities (131, 132). One plausible explanation to these alterations is deafferentation of interconnecting white matter due to widespread axonal damage, causing reduction of metabolic activity and eventually neurocognitive deficits (133, 134).

Although SPECT cannot be independently used in the evaluation of DAI due to limited image resolution and sensitivity, it provides an available and affordable adjunct measure to other anatomical imaging modalities of white matter injury.

Positron Emission Tomography (PET)

Imaging of physiologic and biochemical processes following DAI is also possible by PET, using radiopharmaceutical agents labeled with positron-emitting radioisotopes such as fluorine-18 [^{18}F], carbon-11 [^{11}C], and oxygen-15 [^{15}O]. The [^{18}F]-labeled fluorodeoxyglucose (FDG) PET is widely used in brain imaging to measure local glucose metabolism, and thus regional neuronal activity. Similarly, FDG PET studies of DAI patients have revealed regional hypometabolism in medial frontal lobe structures including the cingulate gyrus (134), findings associated with neuropsychological and cognitive symptoms (134, 135). Additionally, neuroinflammatory alterations can be studied with [^{11}C] PK11195, reflecting microglial activation (136). Using this tracer, widespread neuroinflammation post-TBI was observed in subcortical structures of DAI patients (136, 137). Furthermore, amyloid binding tracers commonly used in Alzheimer's disease have recently established PET as a method for imaging of amyloid-β (Aβ) also in TBI (138, 139). Aβ retention signals were assessed in nine TBI patients, of which four had DAI, and appeared to peak within the first week post-injury (138) and correlate with white matter damage. However, in the chronic stage, Aβ retention signals were also increased with longer time from the initial injury (139).

Future studies will provide knowledge on the topographical distribution and temporal patterns of Aβ deposition following axonal injury, and their relation to the development of neurodegenerative diseases. Although PET scan is a powerful tool which offers superior image resolution, sensitivity, and quantification of regional radioactivity concentrations compared to SPECT (140), its main disadvantage remains the requirement of an on-site cyclotron which considerably increases cost and limits availability.

FLUID AND STRUCTURAL BIOMARKERS

Per definition, biomarkers are molecules measurable in biological fluids and structures given that the measurable value is related to a biological or pathological process in the body (141, 142) (**Table 1**).

Biomarkers may be subdivided into four categories: diagnostic, prognostic, predictive, and pharmacodynamic and can potentially be used to examine injury severity, monitor pathophysiology of injury, explain adaptive and recovery processes, guide management, predict response to treatment and estimate prognosis following DAI/TBI (141–143). Biomarkers can thus be considered a reflection of the mechanisms resulting in axonal injury, where the underlying structural changes are to a large extent related to the activation of the calpain and caspase enzymes. These enzymes belong to the cysteine protease family and play an important role in cell necrosis and apoptosis (144–150). They are activated by calcium influx leading to cytoskeletal disruption including an impaired axoplasmic transport, axonal swelling and eventually axonal transection/lysis (35, 151–154).

Thus, following TBI and in particular following axonal injury, a delayed axonal transection may occur resulting in the release and accumulation of various biomarkers which can be detected in plasma, CSF, and ISF using cerebral microdialysis (MD) (141, 155) and can, therefore, be used for monitoring. Biomarkers reviewed more extensively below are neurofilaments, tau, Spectrin breakdown products (SBDP) and Aβ and are summarized in **Tables 1** and **2**.

NFL

Neurofilaments (NF) are important components of the axonal cytoskeleton, mainly involved in synapses and neurotransmission (156). They represent intermediate neuronal filaments and include three major subunits: neurofilament light (NF-L), neurofilament medium and neurofilament heavy chain (NF-H) (156). The latter becomes phosphorylated (pNF-H), likely by TBI-induced calcium influx, which can alter axonal integrity (156). Of the three subunits, NF-L is rapidly degraded following axonal injury (157) making it a rather sensitive and specific biomarker for the detection of injured axons (5, 141, 158). Following axotomy, phosphorylated neurofilaments (pNF-H) are released in CSF and blood, correlating with injury severity and outcome both in the pediatric and adult population (159, 160).

Neurofilament light fragments can also be identified in both blood and CSF in TBI (143, 161, 162). Following mild and repetitive impacts to the head like those occurring in contact sports such as boxing, American football and ice hockey, increased levels of NF-L may be associated primarily with injury to long, myelinated axons (8, 162–165). Recently, very high levels of NF-L compared to controls were found in 10 patients with impaired level of consciousness following TBI, with the samples taken at least 10 months following injury, results suggesting ongoing axonal degeneration (166).

Increased serum and CSF NF-L levels in TBI patients did also correlate with clinical outcome although without any predictive value for DAI (167). In another study that included 72 patients with severe TBI (of which 33 had DAI), initial NF-L levels independently predicted clinical outcome (168). Additionally, in patients with severe DAI, a 30-fold increase in serum NF-L was recently found (169).

Repeated biomarker sampling during the course of the disease as well as the correlation with advanced neuroimaging is expected to better discern the role of neurofilaments in DAI, their contribution in the pathophysiology of DAI and their prognostic value on outcome.

Tau

Tau is a structural protein with six isoforms in humans and is a normal constituent of axons. Four distinct isoforms of tau are

TABLE 1 | Blood and cerebrospinal fluid (CSF) levels of common axonal injury biomarkers (neurofilament, tau, SBDP and amyloid-β) in clinical TBI.

Reference	Biomarker	*N*	Type of injury	Compartment	Biomarker levels—control group	Biomarker levels—TBI	Major findings
Zurek et al. (159)	pNF-H	49	Pediatric severe TBI (DAI *n* = 9)	Blood	N/A	TBI: 12 (12–1,482) pg/ml DAI: 159 (12–867) pg/ml	Increased levels in DAI
Al Nimer et al. (167)	NF-L	182	Mild: *n* = 15 Moderate: *n* = 39 Severe: *n* = 128 (DAI: *n* = 40)	Blood, CSF	Serum: 7.9 ng/l CSF: 138 ± 31 ng/l	Serum: 400 (181–865) ng/l CSF: 7,026 (2,610–19,204) ng/l	Serum NF-L correlated negatively to outcome in all TBI patients. No predictive value of NF-L on outcome in DAI patients
Ljungqvist et al. (169)	NF-L	9	DAI	Blood	10.8 ± 5.4 pg/ml	347.12 ± 220.65 pg/ml	30-fold increase of NF-L in DAI. NF-L levels were related to DTI parameters
Zetterberg et al. (163)	NF-L	14	Amateur boxers	CSF	≤125 ng/l	845 ± 1,140 ng/l	Increased in boxers, remained elevated at 3 months
Neselius et al. (164)	NF-L	30	Olympic boxers	CSF	135 ± 51 ng/l	532 ± 553 ng/l	Increased in >80% of boxers
Shahim et al. (165)	NF-L	31	Professional ice hockey players	CSF	238 (128–526) pg/ml	410 (230–1,440) pg/ml	Increased levels in players with PCS more than 1 year
Shahim et al. (168)	NF-L	72	Severe TBI (DAI: *n* = 33)	CSF, blood	In CSF not specified Blood: 13 (11–17) pg/ml	In CSF not specified Blood (GCS 6–8): 196 (89–413) pg/ml; (GCS 3–5): 107 (67–190) pg/ml	Increased serum levels in TBI and predicted poor outcome. Similar dynamics in blood and CSF
Shahim et al. (162)	NF-L	49	Amateur boxers (*n* = 14) Professional hockey players (*n* = 35)	Blood	9 pg/ml (IQR 7–14)	Boxers: 22 pg/ml (IQR 18–34) Hockey players: Elevated values compared to controls[b]	Marked increase in boxers 7–10 days after bout. Highest levels in hockey players at 144 h post-concussion
Bagnato et al. (166)	NF-L	10	Severe, persisting DOC following severe TBI	CSF	1,173 pg/ml (670–3,643)	4,458 ng/ml (695–23,000)	Very high levels of NF-L compared to controls suggesting possible ongoing axonal degeneration up to 19 months following severe TBI
Bazarian et al. (182)	c-tau	35	Mild TBI	Blood	N/A	4.85 ± 9.23 ng/ml	C-tau unreliable as a predictor of 3-month outcome
Bulut et al. (177)	t-tau	60	Mild TBI	Blood	86 ± 48 pg/ml	188 ± 210 pg/ml	Levels in high-risk patients (GCS score 14.3 ± 0.73) were significantly higher than in low-risk patients (14.9 ± 0.33)
Shahim et al. (176)	t-tau	28	Concussed professional ice hockey players	Blood	Pre-season: 4.5 pg/ml (0.06–22.7)	Post-concussion: 10.0 pg/ml (2–102)	Peak t-tau immediately post-concussion
Shahim et al. (172)	tau-A, tau-C	28	Concussed professional ice hockey players	Blood	Values are given in graphs (no average)	Values are given in graphs (no average)	No significant increase in tau-A levels but elevated tau-C levels post-concussion compared to pre-season. Tau-A levels correlated with the duration of post-concussive symptoms.
Franz et al. (207)	t-tau	29	Severe TBI (DAI: *n* = 7)	CSF (lumbar, ventricular)	193 pg/ml (16–326), 109 pg/ml (69–159)	1,756 pg/ml (35–5,720)	Increased tau levels early post-TBI; peak in second week
Zetterberg et al. (163)	t-tau, p-tau	14	Amateur boxers	CSF	t-tau: 325 ± 97.7 ng/l p-tau: 46.4 ± 14.5 ng/l	t-tau: 449 ± 176 ng/l p-tau: 37.9 ± 10.2 ng/l	Increased levels of t-tau in boxers after a bout mainly in those who received many or high-impact hits, resolved at 3 months
Neselius et al. (164)	t-tau, p-tau	30	Olympic boxers	CSF	t-tau: 45 ± 17 ng/l p-tau: 23 ± 6 ng/l	t-tau: 58 ± 25 ng/l p-tau: 21 ± 7 ng/l	Increased levels of t-tau in >80% of boxers. Increasing levels during first 6 days, resolved after 14 days

(*Continued*)

TABLE 1 | Continued

Reference	Biomarker	*N*	Type of injury	Compartment	Biomarker levels—control group	Biomarker levels—TBI	Major findings
Oliver et al. (178)	t-tau	19	American football players	Blood	t-tau: 3.7 ± 0.9 pg/ml	t-tau: 3.0 ± 1.2 pg/ml	No difference between players and non-contact swim athletes following a season
Pineda et al. (193)	SBDP	41	Severe TBI (Diffuse TBI/DAI: *n* = 23)	CSF	Arbitrary units	Arbitrary units	SBDP150 elevated up to 24 h, SBDP145 up to 72 h, SBDP after 24 h post-injury
Brophy et al. (194)	SBDP	38	Severe TBI (DAI: *n* = 20)	CSF	Arbitrary units	Arbitrary units	SBDP150 and SBDP145 elevated 24–72 h post-injury, SBDP120 elevated 24–120 h post-injury
Mondello et al. (185)	SBDP	40	Severe TBI (DAI: *n* = 14)	CSF	SBDP145: 0.52 ± 0.22 ng/ml[a] SBDP120: 1.21 ± 0.48 ng/ml[a]	SBDP145:14.42 ± 0.91 ng/ml SBDP120: 6.05 ± 0.28 ng/ml	Higher SBDP145 and SBDP120 in TBI patients, particularly in patients who died
Siman et al. (190)	SNTF	17	Mild TBI	Blood	Arbitrary units	Arbitrary units	Associated with DAI, as evaluated by DTI, and cognitive impairment at 3 months
Siman et al. (191)	SNTF	28	Professional ice hockey players	Blood	Arbitrary units	Arbitrary units	Elevated levels correlated with concussion and delayed return to play
Raby et al. (206)	Aβ40, Aβ42	6	Severe DAI	CSF	Aβ40: 1.59 ± 0.53 ng/mg Aβ42: 0.38 ± 0.2 ng/mg	Aβ40: 0.94 ± 0.08 ng/mg Aβ42 1.17 ± 0.11 ng/mg	Aβ42 increased in CSF by TBI compared to controls, peaked in week 1, declined over next 2 weeks
Franz et al. (207)	Aβ42	29	Severe TBI (DAI: *n* = 7)	CSF [lumbar (*n* = 14), ventricular (*n* = 15)]	DM: 284 pg/ml (172–564) HD: 388 pg/ml (256–768)	167 pg/ml (120–477)	Low CSF levels associated with a poor outcome
Zetterberg et al. (163)	Aβ40, Aβ42	14	Amateur boxers	CSF	Aβ40: 19,400 ± 5,050 ng/l Aβ42: 773 ± 114 ng/l	Aβ40: 19,300 ± 2,740 ng/l Aβ42:858 ± 128 ng/l	Aβ levels not significantly altered
Olsson et al. (204)	Aβ42	28	Severe DAI	CSF, blood	N/A	CSF: peak 129 (60–171) pg/ml (d5–6) Plasma: peak 57 (37–68) pg/ml (d5–6)	Levels increased stepwise, peak day 5–6
Mondello et al. (205)	Aβ42	12	Severe TBI (DAI: *n* = 6)	CSF, blood	CSF: 537.6 pg/ml (350.8–710) Plasma: 7.3 pg/ml (6.1–8.7)	CSF: 105.9 pg/ml (46.0–216.2) Plasma: 17.0 pg/ml (14.7–28.6)	Decreased in CSF and increased in plasma post-TBI
Shahim et al. (165)	Aβ42	31	Professional ice hockey players	CSF	1,094 (845–1,305) pg/ml	1,000 (757–1,040) pg/ml	Lower levels in PCS
Shahim et al. (210)	Aβ40, Aβ42	28	Professional athletes	CSF	Exact values not reported	Exact values not reported	Lower values in athletes with repeated concussions

Articles including patients with diffuse axonal injury (DAI) and mild TBI where axonal biomarkers were measured are presented. Data are given as mean ± SD, median and range as appropriate.

DM, patients with dementia; DOC, disorder of consciousness; HD, patients with headache; ICP, intracranial pressure; IQR, Interquartile range; NF-L, neurofilament- Light; PCS, post-concussion syndrome; pNF-H, phosphorylated neurofilament-heavy; SBDP, spectrin breakdown products; SNTF, spectrin N-terminal fragment; TBI, traumatic brain injury; UCH-L1, ubiquitin carboxy-terminal hydrolase L1; DTI, diffusion tensor imaging.

[a]*145 and 150 kDa αII-spectrin breakdown products.*

usually applied in biomarker studies; total-tau (t-tau), cleaved microtubule-associated tau (c-tau), phosphorylated tau (p-tau), and the recently discovered tau-A (155, 170–172). Tau has been linked to axonal damage following TBI (141, 173). Specifically, the presence of c-tau in CSF is a highly sensitive indicator of axonal injury (35).

In patients with DAI, t-tau and p-tau levels also increase rapidly within hours after injury, especially in CSF (35, 170, 174).

TABLE 2 | Results from cerebral microdialysis (MD) studies of commonly used biomarkers for monitoring axonal injury in clinical DAI.

Reference	Biomarker	*N*	Type of injury	Biomarker levels—control group	Biomarker levels—TBI	Major findings
Magnoni et al. (218)	NF-L	16	Severe TBI[a]	104 pg/ml [0–1,201 (seemingly normal cortex)]	1,555 pg/ml [range 1,152–2,012 (pericontusional)]	Higher levels in focal injury and pericontusional areas than in DAI
Marklund et al. (217)	t-tau	8	Severe TBI[b]	No controls available. Level of detection 75 pg/ml	2,881 ± 1,774 pg/ml (121–6,500)	Higher levels in focal/mixed TBI than in DAI
Magnoni et al. (218)	t-tau	16	Severe TBI[a]	3,469 pg/ml [1,684–8,691 (*n* seemingly normal cortex)]	15,950 pg/ml [11,390–27,240 (pericontusional)]	Higher values in focal injury/pericontusional than in DAI
Magnoni et al. (111)	t-tau	15	Severe TBI[c]	32 pg/ml (detection level)	12,813 pg/ml (4,858–18,744) first 24 h	High initial t-tau levels declined over time, correlated with DTI
Marklund et al. (217)	Aβ42	8	Severe TBI[b]	15.6 pg/ml (detection level)	167 pg/ml (31–295)	Higher levels of Aβ42 in DAI compared to focal/mixed TBI patients
Magnoni et al. (218)	Aβ1-x	16	Severe TBI[a]	1,023 pg/ml [778–1,968 (seemingly normal cortex)]	270 pg/ml [83–417(pericontusional)]	Lower Aβ levels in focal injury/pericontusional than in DAI
Magnoni et al. (111)	Aβ1-x	15	Severe TBI[c]	4.9 and 7.81 pg/ml (detection level)	756 pg/ml (575–1,079) first 24 h	Low initial Aβ levels that rose over time
Helmy et al. (219)	42 cytokines	12	Severe DAI	N/A	N/A	Cerebral production of numerous cytokines, of which 16 peaked at defined time points post-injury[d], was detected
Helmy et al. (222)	42 cytokines	20	Severe DAI	N/A	N/A	Treatment with rhIL1ra influences microglial phenotype as evaluated by MD cytokines

Only MD studies where data is available for DAI patients are included.

Aβ, Amyloid-β; DAI, diffuse axonal injury; DTI, diffusion tensor imaging; IL, interleukin; N/A, non applicable; NF-L, neurofilament light; rhIL1ra, recombinant human interleukin-1 receptor antagonist; TBI, traumatic brain injury.

[a]*Nine were classified as DAI according to Marshall CT classification.*

[b]*Three patients had DAI.*

[c]*Most patients (11/15) had DAI according to Marshall CT classification. No MD catheters were placed in pericontusional areas.*

[d]*These cytokines included IL10, IL12p40, IL12p70, IP10, monocyte chemotactic protein-1, monocyte chemoattractant protein 3 (MCP3), monocyte inflammatory protein 1a (MIP1a), MIP1b, platelet derived growth factor AA (PDGF-AA), transforming growth factor-a (TGF-a) and vascular endothelial growth factor (VEGF).*

Increased CSF levels of t-tau were found in boxers after repetitive head injury, although this increase was modest compared to that of NF-L (163, 164).

The Simoa platform has shown excellent analytical sensitivity for tau in serum (8, 175). Serum c-tau levels are increased but at much lower levels than in CSF and have been used as an indicator of blood–brain barrier damage (35). Compared to off-season levels, serum t-tau levels were elevated in ice hockey players sustaining a concussion, with the highest levels detected immediately after injury (141, 176). In mild TBI, t-tau levels were found to be higher in high-risk patients with greater likelihood for TBI-related complications than in low-risk individuals (177). However, no difference in serum tau levels was recently noted in American football athletes (178).

In DAI, CSF c-tau correlated negatively with the degree of clinical improvement (170, 179, 180). Furthermore, increased serum c-tau levels were associated with poor outcome in patients with mild TBI (181). In contrast, another study found that c-tau is not a reliable predictor for 3-month outcome following mild TBI (182). In concussed professional ice hockey players, the levels of the newly discovered biomarker tau-A correlated with the duration of symptoms post-injury, and may possibly predict return to play (172).

The association of elevated tau levels with axonal damage is well established. Especially in severe DAI, high tau levels are associated with worse outcome. Ongoing and future research efforts need to focus more on its possible correlation with the extent of injury, interaction with other blood and CSF biomarkers, long-term sequelae, and clinical outcome.

Spectrin Breakdown Products

Spectrin is a cytoskeletal protein playing an important role in the cytoskeletal structure and maintenance of plasma membrane (183). In DAI, spectrin is proteolytically cleaved by calpain, resulting in cytoskeletal destruction (184). SBDP are increased in human CSF and blood following severe TBI and may predict injury severity and outcome (5, 185).

In rodents, SBDP are detected within minutes after DAI (186–188). In human TBI, αII-spectrin N-terminal fragment (SNTF) accumulates in injured axons, rises in serum as early as 1 h after mild TBI and correlates with cognitive impairment (141, 189–191). Importantly, SNTF immunoreactive axons have been also identified both in mild and severe TBI (192). Serum SNFT levels were also increased early after concussion in ice hockey players, particularly in more severe injuries (141, 191).

In severe TBI patients, elevated levels of calpain-mediated 150- and 145-kDa SBDP in CSF were found 24–72 h post-injury (193, 194) which were associated with the initial injury severity and 6-month outcome (193). In addition, CSF calpain-mediated SBDP levels correlated positively with the severity of injury, lesion size, and behavioral deficits in severe TBI, suggesting that CSF SBDPs could be used to evaluate the

magnitude of axonal injury and predict functional deficits (193).

Spectrin breakdown products are relatively new biomarkers detected in serum and CSF. Therefore, ample evidence on their significance in the clinical setting is lacking. However, available data suggests that they represent promising molecules in determining the extent of axonal injury and its association to outcome.

Amyloid-β Peptides

Axonal injury in TBI has been characterized by amyloid precursor protein (APP) immunohistochemistry, accumulating at sites of axonal transport failure (8, 195, 196). The presence of APP-positive axonal bulbs and grossly swollen axons are main findings in DAI (195), observed within hours in severe TBI patients (197). However, APP is not a specific diagnostic marker of DAI, since it may also be detected in non-traumatic, ischemic, axonal injury and in multiple sclerosis plaques (35, 198–200). APP co-accumulates with the enzymes necessary for its cleavage to Aβ peptides, such as presenelin-1 and beta-site APP-cleaving enzyme (19, 201, 202). Conversely, notable amount of Aβ has been repeatedly found in axonal bulbs (17, 32, 201–203).

By cleaving APP, the Aβ peptides Aβ40 and Aβ42, the substrates for Aβ aggregates/plaques also observed in Alzheimer's disease, are produced (19). APP and Aβ species are rapidly detectable following TBI in plasma (204, 205), CSF (205–207) and ISF (208, 209). In severe TBI, monomeric Aβ levels in ventricular CSF were increased stepwise until 5–6 days after injury, although not in plasma (204). Conversely, a more recent study using an ultrasensitive digital immunoassay evaluating 12 severe TBI patients of which 6 had DAI, reduced CSF levels of Aβ42 direct after injury with lower levels in patients who died 6 months post-injury were observed. In the same study, plasma levels were increased with lower levels detected in surviving patients (205). The differences in analytical methods may partly explain the discrepancy in the results between these studies. Additionally, the latter study also included patients with focal TBI, although no difference in Aβ levels was observed between TBI subtypes (205). Similarly, lower CSF levels of Aβ40 and Aβ42 were recently detected in professional athletes following concussions (165, 210).

An increased interest in soluble intermediary Aβ oligomers/protofibrils as the pathogenic form of Aβ has emerged since they are likely to contribute to the development of Alzheimer's disease (211, 212). Aβ oligomers have been detected in lumbar CSF from severe TBI patients, were elevated in patients with poor neurological outcomes and were negatively correlated to CSF Aβ42 (213). Therefore, it is plausible that aggregation of Aβ into oligomers may explain the reduced levels of CSF Aβ seen in TBI. However, the corresponding brain tissue levels of soluble intermediary Aβ species and their role in human DAI remains to be established. In addition, these potentially neurotoxic species could represent a pathophysiologic link between DAI and Alzheimer disease-like dementia.

The association between TBI and the development of neurodegenerative diseases, in particular Alzheimer's disease, has been repeatedly demonstrated (24, 214). Longitudinal monitoring of Aβ dynamics may provide further knowledge of neurodegenerative processes following DAI. Aβ42 levels, which can be monitored in both CSF and blood are likely the most promising biomarker from the amyloid family for detecting the extent and severity of DAI. In addition, specific monitoring of potentially neurotoxic oligomeric and protofibrillar Aβ species will become possible using newly developed antibody-based PET imaging (215). This will further increase the understanding of the potential link between DAI and neurodegeneration.

Biomarkers and Cerebral Microdialysis

Cerebral MD is a neurocritical care monitoring technique predominantly used in patients with severe TBI and subarachnoid hemorrhage (**Table 2**). Its main advantage is that it allows continuous neurochemical monitoring of factors located in the extracellular, interstitial fluid (216).

Using MD on 8 severe TBI patients, particularly high ISF Aβ42 values were found in the three DAI patients (217). In another MD study, in nine DAI patients the initial Aβ levels inversely correlated with tau levels in ISF (218), suggesting that low Aβ levels in regions with elevated tau may be due to reduced synaptic activity after axonal injury (218).

Tau was also evaluated by MD in eight severe TBI patients (217). Although mean t-tau levels were clearly above the detection limit in the first days after injury, patients with focal/mixed injury ($n = 5$) had lower levels compared to those with DAI ($n = 3$). Conversely, in a previous MD study, higher tau values were observed pericontusionally in focal TBI patients when compared to tau levels obtained from DAI patients with the MD catheter placed in structurally normal frontal cortex. Early tau levels were inversely correlated with the initial Aβ levels (155, 218). In this study, NF-L levels were also higher in pericontusional tissue (218). Further, in a study of 15 patients with severe TBI (11/15 had DAI), initially high t-tau levels in ISF declined over time and a correlation with DTI and reduced brain white matter integrity in the region of MD sampling was observed, suggesting that increased tau levels reflected axonal injury (111).

The cytokine response was evaluated by MD in patients with severe DAI suggesting that cytokine production is highly compartmentalized with significant differences between brain parenchymal and systemic concentrations (219–222). Several cytokines are produced in different phases of the inflammatory response (220). It has been also shown that in DAI patients, treatment with an interleukin receptor-1 antagonist increased microglial activation, altering the cytokine profile to one consistent with an M1 microglial phenotype, providing proof of concept that an anti-inflammatory treatment administered systemically can alter cerebral cytokine productions in human TBI (222). These data suggested that the patterns of cytokine release in ISF are promising targets for biomarker research in DAI.

Following DAI, markers analyzed in MD samples indicating acute and chronic neuroinflammation may potentially be used to guide treatment, as measures for pharmacological response, and/or for tissue outcome. Specifically, MD may aid in detecting factors related to the progression of the disease and in the understanding of the pathophysiology of axonal injury. Therefore, it is likely that data from MD, in combination with widely used

measures such as ICP-CPP guided monitoring and protocols, will contribute to the understanding of the pathophysiology of DAI and potentially aid in evaluating novel pharmacological treatments.

However, MD is time consuming, usually used for low-molecular weight molecules and frequently characterized by high variability and lack of standardization. In addition, MD remains a predominantly focal measurement technique. To date, there is insufficient data arguing for MD to be used as a clinical decision tool for DAI patients and should rather be considered an integral part of the multimodality monitoring during neurocritical care as well as a research tool.

Other Biomarkers

There are numerous additional biomarkers associated to CNS injury that can potentially be related to axonal injury. Examples of such biomarkers are Glial Fibrillary Acidic Protein, ubiquitin carboxy-terminal hydrolase L1 myelin basic protein, microtubule-associated protein 2, protein S-100B and neuron-specific enolase, among others (5, 35, 141–143, 223–228). Especially the astrocytic protein S-100B is a promising biomarker across all injury severities, with higher S-100B levels observed in focal compared to diffuse TBI (229, 230). Moreover, it has been shown that S-100B correlates with Marshall CT classification scores (231). Furthermore, following TBI, neuroinflammation may as previously noted play an important role as a key secondary injury factor (141, 232). Specifically, it has been repeatedly shown both in the experimental and clinical setting that cytokines such as Tumor Necrosis Factor-α and Interleukins (ILs) 1β, 6, 8, and 10 are increased following TBI both in blood and CSF (233).

Since the above mentioned biomarkers are not specific for axonal injury, at present, their elevations in CSF or serum should be interpreted with caution from both the diagnostic and predictive perspective with regards to DAI.

Limitations of Biomarkers

Although considerable progress has been made in the recent years in research, the quest for TBI-specific biomarkers continues. The currently used biomarkers commonly have different specificity- and/or sensitivity, limited availability for bedside analysis and use in daily practice as well as variable half-lives. Moreover, some can also be released from other organ systems during different disease or injury processes.

Biomarkers obtained from CSF and ISF are theoretically considered better and more reliable sources compared to blood biomarkers. Therefore, it is preferable to obtain samples from these compartments whenever possible. However, in particular in mild and moderate TBI, there is generally no clinical indication for CSF and, for obvious reasons, ISF sampling by invasive means. On the other hand, blood samples are easily accessible in almost every TBI patient.

Nevertheless, important issues relevant to TBI-related blood biomarkers include their relatively low concentrations, proteolytic degradation, the requirement of carrier proteins and the different permeability across the blood–brain barrier for certain biomarkers (141).

Standardization and validation of biomarker levels are other important issues since different methods of analysis, preparation and sample quality can provide different results among laboratories and centers which can cause problems during the interpretation and comparison of results (234). As the field of biomarker research is expanding, CNS sensitivity and/or specificity increases their importance. Analysis of many currently used biomarkers requires specialized research laboratories which are not available on a daily basis. Additionally, difficulties may be encountered when attempting to assess biomarker half-time, especially in those that are continuously released from the brain following injury and in those with complex elimination or degradation mechanisms (230).

RECOMMENDATIONS FOR MONITORING AXONAL INJURY

1. ICP and CPP monitoring as well as ICP-CPP guided therapy are advised in all severe TBI patients with suspected axonal injury and decreased level of consciousness especially in the initial post-injury phase.
2. Conventional MRI scan sequences such as FLAIR, DWI, and SWI should be considered in the first post-injury period following TBI to detect and confirm the presence of DAI.
3. Advanced MRI techniques such as DTI and MRS are useful modalities for further delineation of axonal damage in TBI, particularly in the subacute and chronic phase.
4. Due to high cost and limited availability, PET scanning is recommended solely as a valuable research tool although not to date in clinical management.
5. Biomarkers specific for axonal injury can be analyzed in blood and CSF from the acute to chronic post-injury period in TBI, aiming to aid in the understanding of the axonal injury process, follow the course of the disease, monitor for possible deterioration, estimate the extent of axonal injury and aid in prognostication.
6. Repeated clinical examinations and neuropsychological tests can provide invaluable information on the extent of injury, prognosis and for monitoring possible recovery or exacerbation of cognitive functions and mental status.

CONCLUSION

Although axonal injury has traditionally been associated with an impaired level of consciousness and poor prognosis, patients with confirmed axonal damage can achieve a good clinical outcome. Using advanced neuroimaging, axonal injury is increasingly recognized also in mild TBI or sports-related concussions. Many tenets of the pathophysiology of axonal injury are being elucidated through major efforts in basic science and medical research. Nonetheless, it remains an exceedingly complex subtype of TBI with many unknown secondary pathological processes. Since the secondary injury cascades are continuing for a considerable time post-injury, monitoring is critically important for clinical as well as research purposes. Advanced imaging techniques such as MRS, DTI and PET show promise in better identifying and quantifying axonal injury and its importance for patient outcome. In addition,

both invasive and non-invasive neurocritical care techniques are becoming increasingly important in monitoring axonal injury. Numerous biomarkers with, plausibly, high specificity for axonal damage have been and are being developed. This evolving field of TBI research is promising for the development of bedside, rapid analysis kits for small-volume body fluids. When these novel biomarkers are available for routine use as monitoring tools for axonal injury, they may in the future aid in the detection and prevention of secondary axotomy and atrophy of white matter tracts. They may also be used as secondary outcome measures in DAI, assist in the development of novel therapies, guide treatment, and monitor treatment response. Finally, the short-and long-term monitoring options for axonal pathology and its progression described in this review may become crucial for the prevention of neurodegeneration at the chronic stage in DAI.

AUTHOR CONTRIBUTIONS

Study concept and design, drafting of the manuscript, analysis and interpretation of data, and critical revision of the manuscript: PT, SAH, and NM. Acquisition of data: PT and SAH. Study supervision: NM.

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REVIEW
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Cerebrospinal Fluid and Microdialysis Cytokines in Severe Traumatic Brain Injury: A Scoping Systematic Review

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Objective: To perform two scoping systematic reviews of the literature on cytokine measurement in: 1. cerebral microdialysis (CMD) and 2. cerebrospinal fluid (CSF) in severe traumatic brain injury (TBI) patients.

Methods: Two separate systematic reviews were conducted: one for CMD cytokines and the second for CSF cytokines. Both were conducted in severe TBI (sTBI) patients only.

Data sources: Articles from MEDLINE, BIOSIS, EMBASE, Global Health, Scopus, Cochrane Library (inception to October 2016), reference lists of relevant articles, and gray literature were searched.

Study selection: Two reviewers independently identified all manuscripts utilizing pre-defined inclusion/exclusion criteria. A two-tier filter of references was conducted.

Data extraction: Patient demographic and study data were extracted to tables.

Results: There were 19 studies identified describing the analysis of cytokines *via* CMD in 267 sTBI patients. Similarly, there were 32 studies identified describing the analysis of CSF cytokines in 1,363 sTBI patients. The two systematic reviews demonstrated: 1. limited literature available on CMD cytokine measurement in sTBI, with some preliminary data supporting feasibility of measurement and associations between cytokines and patient outcome. 2. Various CSF measured cytokines may be associated with patient outcome at 6–12 months, including interleukin (IL)-1b, IL-1ra, IL-6, IL-8, IL-10, and tumor necrosis factor 3. There is little to no literature in support of an association between CSF cytokines and neurophysiologic or tissue outcomes.

Conclusion: The evaluation of CMD and CSF cytokines is an emerging area of the literature in sTBI. Further, large prospective multicenter studies on cytokines in CMD and CSF need to be conducted.

Keywords: cytokines, traumatic brain injury, brain injury, systematic review, microdialysis, cerebrospinal fluid

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INTRODUCTION

Neuroinflammation after traumatic brain injury (TBI) is postulated to be a key driver of secondary brain injury in the acute/subacute phase after injury (1, 2). Upregulation of various components of the inflammatory cascade have been associated with lesion expansion (3), cerebral edema (4), derangements in neural transmission (5), and subsequent tissue death (6) in animal models of stroke and TBI. In humans, the inflammatory process post-TBI has been of interest, since its therapeutic modulation can potentially lead to amelioration of pathophysiology, tissue salvage, and improved patient outcomes (7, 8). Serum cytokine levels are easily measured in TBI patients, and elevation in pro-inflammatory cytokines have been associated with worse patient outcome (9, 10). However, systemic cytokine levels can be confounded by extracranial pathology and variable blood–brain barrier leak of centrally derived mediators. Measurement of cerebral levels of cytokines provides a more direct metric of neuroinflammation following TBI, but, to date, the measurement of cerebral microdialysis (CMD) (11–29) and cerebrospinal fluid (CSF) (30–65) cytokines have been limited to small studies.

The goal of this study was to produce a scoping systematic review of the literature on both CMD and CSF cytokines in severe TBI (sTBI). Our hope was to produce a comprehensive overview of the literature on this emerging topic.

METHODS

Two separate scoping systematic reviews were conducted, using the methodology outlined in the Cochrane Handbook for Systematic Reviewers (66). Data were reported following the preferred reporting items for systematic reviews and meta-analyses (67). The review questions and search strategy were decided upon by the primary author (Frederick A. Zeiler) and supervisors (Adel Helmy and David K. Menon).

This manuscript was conducted in concert with a similar review on cytokines in CMD and CSF for aneurysmal subarachnoid hemorrhage (SAH) patients.

Search Question and Population of Interest

Given that two separate systematic reviews were conducted, one for CMD cytokines and the other for CSF cytokines, two distinct questions were posed. The limited literature on CMD cytokines identified through a preliminary search of PubMed led us to conduct a scoping review for the CMD cytokine search. We attempted to identify all studies in this area to date, and all articles describing microdialysis cytokine measures in humans with sTBI included in our review in order to provide a comprehensive overview of this emerging area of literature. The key question for this part of the review was:

- What literature has been published on CMD of cytokines in sTBI?

The larger literature base for CSF cytokines in TBI led us to narrow our question for this scoping review, focusing on relevant outcomes (see below). The questions posed for this scoping systematic review was:

- Is there literature to suggest an association between CSF cytokine measures in sTBI and patient outcome, neurophysiologic outcome, or tissue outcome?

For the CSF cytokine review, the primary outcome measures were documented association between CSF cytokine levels and: patient outcome, neurophysiologic outcome (as measured *via* intensive care unit (ICU)-based monitoring; intracranial pressure (ICP)/cerebral perfusion pressure (CPP), brain tissue oxygen monitoring ($PbtO_2$), thermal diffusion assessment of cerebral blood flow (CBF), transcranial Doppler (TCD) measure of cerebral blood flow velocity (CBFV), any neuroimaging based assessment of CBF/perfusion, and electrophysiology), and tissue outcome [as assessed on follow-up neuroimaging by either computed tomography (CT) or magnetic resonance imaging]. Any outcome score or mention of morbidity/mortality within the studies was deemed acceptable for documentation of patient outcome. Secondary outcome measures were complications associated with CSF monitoring of cytokines.

The list of included cytokines in CMD or CSF included: interleukin (IL)-1a, IL-1b, IL-1ra, IL-2, sIL-2ra, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-12p70, IL-13, IL-14, IL-15, IL-16, IL-17, inducible protein (IP)-10, eotaxin, tumor necrosis factor (TNF), interferon gamma (INF-g), monocyte chemoattractant proteins, macrophage inflammatory proteins (MIPs), transforming growth factor (TGF), nerve growth factor (NGF), brain-derived neurotrophic factor, glial-derived neurotrophic factor, soluble tumor necrosis factor receptor (sTNFR), granulocyte macrophage colony stimulating factor, soluble FAS, soluble vascular cell adhesion molecule (sVCAM)-1, and soluble intracellular adhesion molecule (sICAM)-1, platelet-derived growth factor, regulated on activation, normal T cell expressed and secreted (RANTES), macrophage-derived chemokine (MDC), fms-like tyrosine kinase 3 (Flt3), Fractalkine, and fibroblast growth factor receptor.

Inclusion/Exclusion Criteria

CMD Cytokine Review

Inclusion criteria were: all studies including human subjects with sTBI (GCS 8 or less), any study size, any age category, CMD analysis for cytokines, and mention of any outcome (patient based or otherwise). Exclusion criteria were: non-English studies and animal studies.

CSF Cytokine Review

Inclusion criteria were: all studies including human subjects with sTBI (GCS of 8 or less), studies with 10 or more patients, any age category, CSF analysis for cytokines, and documentation either: patient functional outcome, neurophysiologic outcome, or tissue outcome in relation to CSF cytokine measures. Exclusion criteria were: non-English studies, animal studies, and studies of less than 10 patients. Non-English studies were excluded given the small number identified.

Search Strategies

MEDLINE, BIOSIS, EMBASE, Global Health, SCOPUS, and Cochrane Library from inception to October 2016 were searched

using individualized search strategies. The search strategy for the CMD scoping systematic review using MEDLINE can be seen in Appendix A in Supplementary Material, with a similar search strategy utilized for the other databases. Further, the search strategy for the CSF scoping systematic review using MEDLINE can be seen in Appendix B in Supplementary Material, with similar strategies employed for the other databases.

In addition, we surveyed relevant meeting proceedings for the last 5 years looking for ongoing and unpublished work based on cytokine analysis *via* CMD or CSF in sTBI patients. The meeting proceedings of the following professional societies were searched: Canadian Neurological Sciences Federation, American Association of Neurological Surgeons, Congress of Neurological Surgeons, European Neurosurgical Society, World Federation of Neurological Surgeons, National Neurotrauma Society, American Neurology Association, American Academy of Neurology, European Federation of Neurological Science, World Congress of Neurology, Society of Critical Care Medicine, Neurocritical Care Society, European Society for Intensive Care Medicine, World Federation of Societies of Intensive and Critical Care Medicine, American Society for Anesthesiologists, World Federation of Societies of Anesthesiologist, Australian Society of Anesthesiologists, International Anesthesia Research Society, Society of Neurosurgical Anesthesiology and Critical Care, Society for Neuroscience in Anesthesiology and Critical Care, Japanese Society of Neuroanesthesia and Critical Care, International NeuroTrauma Society, International Brain Injury Association, and the College of Intensive Care Medicine Annual Scientific Meeting (CICMASM—Australia).

Finally, reference lists of any review articles on CSF or CMD cytokines were reviewed for any missed relevant studies.

Study Selection

Utilizing two reviewers, a two-step review of all articles returned by our search strategies was performed. First, the reviewers independently (Frederick A. Zeiler and Eric Peter Thelin) screened titles and abstracts of the returned articles to decide if they met the inclusion criteria. Second, full text of the chosen articles was then assessed to confirm if they met the inclusion criteria and that the primary outcomes of interest were reported in the study (Frederick A. Zeiler and Eric Peter Thelin). Any discrepancies between the two reviewers were resolved by a third reviewer if needed (Adel Helmy or David K. Menon).

Data Collection

Data were extracted from the selected articles and stored in an electronic database. Data fields included: patient demographics, type of study, article location, number of patients, CMD/CSF substrate measured, CMD/CSF measurement details (probe tissue location, sampling frequency), outcome measure described (patient, neurophysiologic, tissue), association between CMD/CSF cytokine measure to outcome, and complications. All extracted data can be found in **Tables 1** through **4**, with study designs in **Tables 1** and **2**, and study outcomes in **Tables 3** and **4**.

Bias Assessment

As the goal of this review was to produce a systematically conducted scoping review of the available literature on CMD and CSF cytokine measures in sTBI, formal bias assessment was not done. Our desire was to produce a comprehensive overview of the current literature on the topic of CMD/CSF cytokines in sTBI. Formal evidence grading was not conducted (given the limited and heterogenous literature body), and thus we deemed formal bias risk assessment unnecessary for this emerging area of literature, which clearly suffers from standard biases associated with new areas of clinical research.

Statistical Analysis

A meta-analysis was not performed in this study due to the heterogeneity of data and study design within the articles identified.

RESULTS

Search Strategy Results

CMD Cytokine Search

Results of the search strategy for CMD cytokines in sTBI is shown in the flow diagram in **Figure 1**. In total, 259 articles were identified, with 255 from the database and 4 from meeting proceeding sources. After removal of the duplicates, there were 144 articles left for assessment in the first filter of title and abstract. Thirty-seven articles passed the first filter, requiring acquisition of the full manuscript to assess inclusion eligibility. After assessing the full manuscripts, 19 articles were deemed eligible for final inclusion in the scoping systematic review. No articles were added from the reference sections of either review papers or the parent manuscripts included in the systematic review.

CSF Cytokine Search

The search strategy flow diagram for the CSF cytokine scoping systematic review is shown in **Figure 2**. Overall, 3,218 articles were identified, with 3,214 from the database search and 4 from published meeting proceedings. There were 1,317 duplicates removed, leaving 1,901 references to review in the first filter. Applying the inclusion/exclusion criteria to the title and abstract of these articles, 105 manuscripts were selected for review of the full article. One additional reference was added from the reference sections of review papers. During the second filter of the full manuscript, 36 met the final inclusion criteria for the scoping systematic review. Remaining articles were excluded due to non-relevance.

Patient/Study Demographics

CDM Cytokine Review

Of the 19 articles included in the CMD cytokine portion of the systematic review (11–19), 15 were formal manuscript publications (14–24, 26–29) and 4 were meeting abstract publications (11–13, 25). There were 13 prospective studies (13–16, 18–21, 25–29), with 12 prospective observational studies (13–15, 18–21, 25–29) and 1 prospective randomized control trial (16). Four studies were retrospective case series or database reviews (11, 20, 22, 23). Finally, two studies were of "unknown" study design given a lack of information available within the methods (12, 24).

The study population described in CMD cytokine papers was generally poorly characterized sTBI patient populations, undergoing various ICU and surgical therapies for their heterogeneous

TABLE 1 | CMD cytokine study characteristics and patient demographics.

Reference	Number of patients	Study type	Article location	Mean age (years)	Patient characteristics	Primary and secondary goal of study
Cederberg et al. (11)	7	Retrospective case series	Meeting abstract	Unknown "children"	Severe TBI; 3 underwent DC	Primary: to compare CMD cytokines to common CMD measures, $PbtO_2$, and ICP Secondary: none mentioned
Figaji et al. (12)	5	Unknown	Meeting abstract	Unknown "children"	Severe TBI	Primary: to compare CMD cytokine and other CMD measures Secondary: none mentioned
Guilfoyle et al. (13)	12	Prospective observational	Meeting abstract	Unknown "adults"	Severe TBI	Primary: to compared CMD cytokine measures in healthy vs. peri-lesional tissue Secondary: none mentioned
[a]Helmy et al. (14)	12	Prospective observational	Manuscript	Unknown "adults"	Severe TBI	Primary: to perform a principle component analysis of CMD cytokines to determine cytokine patterns and temporal profiles Secondary: none mentioned
[a]Helmy et al. (15)	12	Prospective observational	Manuscript	Unknown "adults"	Severe TBI	Primary: 1. To compare crystalloid vs. albumin perfusate in CMD cytokine recovery. 2. To compare the cytokine profile in sTBI Secondary: not specified
[b]Helmy et al. (16)	20	Prospective RCT	Manuscript	38.9 years (range: 18–61 years)	Severe diffuse TBI; randomized to subcutaneous rhIL-1ra	Primary: 1. To provide safety data in a randomized fashion on rhIL-1ra in sTBI 2. To describe the impact of rhIL-1ra on CMD cytokine profiles Secondary: none mentioned
[b]Helmy et al. (17)	20	Retrospective database analysis	Manuscript	38.9 years (range: 18–61 years)	Severe diffuse TBI; randomized to subcutaneous rhIL-1ra	Primary: to retrospectively analyze RCT data on rhIL-1ra administration, to better delineate the temporal change in cytokine profiles Secondary: none mentioned
Hillman et al. (18)	9 (10 total, but failed CMD catheter in 1)	Prospective observational	Manuscript	Unknown	Severe brain injury (undisclosed number of aSAH and sTBI patients)	Primary: to evaluate newer microdialysis catheters and their ability to measure various CMD macromolecules (including IL-6) vs. older catheters. Varied perfusates were also analyzed Secondary: none mentioned
Hillman et al. (19)	7 with TBI (14 total; mixed injury sources)	Prospective observational	Manuscript	Unknown	sTBI—5 requiring "surgery"	Primary: to determine the CMD cytokine patterns in TBI Secondary: none mentioned
Hutchinson et al. (20)	15	Prospective observational	Manuscript	41 years (range: 17–68 years)	Severe TBI	Primary: to determine the feasibility of measures IL-1a, IL-1b, and IL-1ra in CMD samples Secondary: correlation of cytokine to ICP, CPP, and patient outcome
Mellergard et al. (21)	7 (total 38 patients; only 7 with TBI)	Prospective observational	Manuscript	Unknown	Severe TBI	Primary: to evaluate CMD cytokine profiles immediately after insertion of the CMD catheter Secondary: none mentioned
[c]Mellergard et al. (22)	57 (total 145 patients; only 57 with TBI)	Retrospective case series	Manuscript	Unknown	Severe TBI	Primary: to determine the CMD cytokine responds to TBI Secondary: none mentioned
[c]Mellergard et al. (23)	57 (total 145 patients; only 57 with TBI)	Retrospective case series	Manuscript	Unknown	Severe TBI	Primary: to determine the CMD cytokine responds to TBI Secondary: none mentioned
Mellergard et al. (24)	69	Unknown	Manuscript	45.9 years (range: unknown)	Severe TBI	Primary: to determine if there is age-related difference in CMD cytokines Secondary: none mentioned
Mondello et al. (25)	6	Prospective observational	Meeting abstract	Unknown	Severe TBI	Primary: to evaluate the temporal profile of CMD and CSF cytokines in TBI Secondary: none mentioned

(*Continued*)

TABLE 1 | Continued

Reference	Number of patients	Study type	Article location	Mean age (years)	Patient characteristics	Primary and secondary goal of study
Parez-Barcena et al. (26)	16	Prospective observational	Manuscript	31.8 years (range: 16–65 years)	Severe diffuse TBI	Primary: to determine the cytokine profiles in severe diffuse TBI patients Secondary: to determine the correlation between cytokines and ICP, $PbtO_2$, and CT changes
Roberts et al. (27)	8	Prospective observational	Manuscript	43.4 years (range: unknown)	Severe TBI	Primary: to measure the blood/CSF/CMD MMP and cytokine response post-TBI Secondary: correlation to neurologic exam, ICP, $PbtO_2$, GOS at discharge
Winter et al. (28)	3	Prospective observational	Manuscript	Unknown	Severe TBI	Primary: to describe the technique of cytokine measurement *via* CMD Secondary: describe cytokine patterns in TBI
Winter et al. (29)	14	Prospective observational	Manuscript	43.1 years (range: 21–77 years)	Severe TBI	Primary: to evaluate the changes in CMD cytokines post-TBI Secondary: correlation to patient outcome

TBI, traumatic brain injury; sTBI, severe TBI; aSAH, aneurysmal subarachnoid hemorrhage; DC, decompressive craniectomy; CMD, cerebral microdialysis; RCT, randomized control trial; ICP, intracranial pressure; CPP, cerebral perfusion pressure; CSF, cerebrospinal fluid; LPR, lactate:pyruvate ratio; CT, computed tomography; $PbtO_2$, partial pressure of oxygen in brain tissue; MMPs, matrix metalloproteins, IL, interleukin; a, alpha; b, beta; ra, receptor anatogonist; rh, recombinant human.

[a]*Same patient population reported in both Helmy et al. (14) and Helmy et al. (15).*

[b]*Same patient population described in Helmy et al. (16) and Helmy et al. (17).*

[c]*Same patient population reported in both Mellergard et al. (22) and Mellergard et al. (23).*

intracranial pathology (11–15, 18–25, 27–29). Three studies focused on only those patients with imaging defined "diffuse" brain injury, without extra-axial or large focal intraparenchymal lesions (16, 17, 26).

A total of 267 unique patients with sTBI were described across the 19 studies included in the CMD cytokine review. Thirty-six patients were "diffuse" sTBI only (16, 17, 26), with the remaining being unspecified heterogeneous sTBI pathology. We believe that some of the studies included within this portion of the review may contain duplicate patient information, as marked in **Tables 1** and **3**. Multiple publications from the same research groups likely were conducted on the same patient populations, yielding unique and separate manuscripts on the same group of patients. Though we must acknowledge it was difficult to determine, in some circumstances, whether CMD cytokine analysis was being conducted on new patient groups or existing banked samples from previous prospective studies. With that said, our goal for the CMD cytokine scoping review was to provide an overview of all available literature in the area, hence we have included all published papers on CMD cytokines in sTBI within this review.

CSF Cytokine Review

Of the 36 articles included in the CSF cytokine systematic review (20–65), 32 were formal manuscript publications (30–36, 39, 40, 42–61, 63–65) and 4 were meeting abstract publications (37, 38, 41, 62). There were 34 prospective studies, all being observational studies (30–61, 64, 65). One study was a retrospective case series (63). Finally, one study had insufficient information to determine the design (62).

The populations described with in the CSF cytokine studies were almost all sTBI patients with unspecified heterogeneous injury patterns. Three studies documented the inclusion of both moderate-severe patients within the methods (39, 53, 62). We were unable to separate the moderate and sTBI patients within these studies, hence they were all included in the final descriptive statistics.

A total of 1,363 patients were described across all studies included in the CSF cytokine systematic review. The mean age for each study cohort varied significantly across studies. Twenty-one studies included pediatric patients within their studies, either as the primary population of interest or included with adult patients (31–35, 42, 44, 47–50, 52, 54, 55, 57, 59, 60, 63–65). Therapies received by these patients while in the ICU varied significantly, with profound heterogeneity in treatment provided. Details surrounding patient cohort, study design, and concurrent therapies can be found in **Tables 2** and **4**. We made substantial efforts to exclude duplicate patient data across studies. However, given that many of the papers came from centers of excellence for TBI research, some of the patient data may be cross reported in multiple studies. This could reduce the total overall number of unique patients. It was impossible based on the information provided within the parent studies to tease out all patients which were reported more than once.

Cytokine Measurement Technique

CMD Cytokine Review

Location of the CMD catheter was the following: mixed healthy/peri-lesional tissue in six studies (11, 13, 15, 21–23), peri-lesional in six studies (14, 16–19, 28), healthy tissue in two studies (27, 29), and unknown tissue location in five studies (12, 20, 24–26). Some studies utilized paired microdialysis catheters, one in healthy and one in peri-lesional tissue (13, 15, 22, 23). One study evaluated two catheters in one location (18). Analysis interval for CMD samples was as follows: every 6 h in 12 studies (14–24, 27), every 8 h in 1 study (26), every 3 h in 2 studies (28, 29), and unspecified in 4 studies (11–13, 25). The duration

TABLE 2 | CSF cytokine study characteristics and patient demographics.

Reference	Number of patients	Study type	Article location	Mean age (years)	Patient characteristics	Primary and secondary goal of study
Patient functional outcome						
Abboud et al. (30)	31	Prospective observational	Manuscript	31.6 years (range: unknown)	Severe TBI	Primary: to describe the correlation between CSF cytokine profiles and outcome at 6 and 12 months Secondary: none mentioned
Bell et al. (31)	15	Prospective observational	Manuscript	6.1 years (range: 0.1–16 years)	Severe TBI	Primary: to determine the relationship between IL-6 and IL-10 with patient outcome Secondary: to compare CSF cytokine levels to non-TBI control subjects (n = 20)
Chiaretti et al. (32)	29	Prospective observational	Manuscript	9.7 years (range: 1.3–15.6 years)	Severe TBI	Primary: to determine the association between IL-6 and patient outcome Secondary: to determine the correlation between IL-6 and NGF in CSF. Also to compare to non-TBI control patients (n = 31)
Chiaretti et al. (33)	27	Prospective observational	Manuscript	8.6 years (range: 1.3–15.6)	Severe TBI	Primary: to determine the association between IL-1b, IL-6, NGF, BDNF, and GDNF with patient outcome Secondary: none mentioned
Chiaretti et al. (34)	14	Prospective observational	Manuscript	7.8 years (range: 0.3–15.6 years)	Severe TBI	Primary: to determine the relationship between IL-1b and IL-6 with patient outcome Secondary: to compared cytokine expression to obstructive hydrocephalus controls
Hans et al. (35)	11	Prospective observational	Manuscript	36.7 years (range: 16–67)	Severe TBI	Primary: to determine the association between IL-6 and sIL-6R to patient outcome Secondary: to compare these CSF cytokine levels to those in plasma
Hayakata et al. (36)	53	Prospective observational	Manuscript	34–49 years	Severe TBI	Primary: to determine the association between TNF-a, IL-1, IL-6, IL-8, and IL-10 with patient outcome Secondary: to determine the association between cytokines and S100B expression in CSF. Also compare cytokines to ICP
Jamil et al. (37)	61	Prospective observational	Meeting abstract	Unknown "adults"	Severe TBI	Primary: to determine the relationship between acute measures of CSF cytokines and PTD at 6 and 12 months Secondary: none mentioned
Juengst et al. (38)	25	Prospective observational	Meeting abstract	Unknown "adults"	Severe TBI	Primary: to determine the association between acute cytokine levels and apathy at 6 and 12 months post-injury Secondary: none mentioned
Juengst et al. (39)	37	Prospective observational	Manuscript	"Adults" Unclear overall mean age	Moderate–severe TBI	Primary: to determine the relationship between TNF-a and disinhibition/suicidality post-TBI Secondary: compare levels in CSF and serum to healthy controls (n = 15)
Juengst et al. (40)	50	Prospective observational	Manuscript	31.3 years (range: unknown)	Severe TBI	Primary: to determine the relationship between acute CSF cytokine profiles and the risk of PTD at 6 and 12 months post-injury Secondary: none mentioned
Kirchhoff et al. (41)	23	Prospective observational	Meeting abstract	Unknown	Severe TBI	Primary: to determine the IL-10 response in CSF in TBI patients. Also determine the relationship to outcome. Secondary: compared CSF in TBI to elective surgical patients (n = 10)
Kossmann et al. (42)	22	Prospective observational	Manuscript	41 years (range: 17–73)	Severe TBI	Primary: to determine the relationship between CSF IL-6 and NGF. Also determine the association to patient outcome. Secondary: compare IL-6 and NGF in controls (n = 3)

(*Continued*)

TABLE 2 | Continued

Reference	Number of patients	Study type	Article location	Mean age (years)	Patient characteristics	Primary and secondary goal of study
Kumar et al. (43)	114	Prospective observational	Manuscript	Unclear overall mean age	Severe TBI	Primary: to determine the relationship of IL-6 in CSF to serum values and patient outcome Secondary: compare CSF levels in non-TBI controls (n = 23)
Kumar et al. (44)	111	Prospective observational	Manuscript	Unknown (range: 16–75)	Severe TBI	Primary: to utilize PCA to determine clusters of cytokines associated with patient outcome Secondary: to determine a temporal patter of cytokine clusters and relationship to outcome
Kushi et al. (45)	22	Prospective observational	Manuscript	45 years (range: unknown)	Severe TBI	Primary: to compare CSF and Serum IL-6/IL-8 levels and determine the association to patient outcome Secondary: none mentioned
Nwachuku et al. (46)	32	Prospective observational	Manuscript	31 years (range: unknown)	Severe TBI	Primary: to determine the association between various CSF cytokines and patient outcome Secondary: none mentioned
Santarseiri et al. (47)	91	Prospective observational	Manuscript	35.8 years (range: 16–73)	Severe TBI	Primary: to identify CSF cytokines associated with patient outcome. Also determine association between cytokines and neuroendocrine cortisol function Secondary: none mentioned
Shiozaki et al. (48)	35	Prospective observational	Manuscript	39 years (range: 14–77 years)	Severe TBI	Primary: to determine the association between CSF cytokine profiles and patient outcome Secondary: to determine the association between cytokines and ICP
Singhal et al. (49)	36	Prospective observational	Manuscript	34.4 years (range: 17–68 years)	Severe TBI	Primary: to determine the association between cytokines and electrophysiologic/functional patient outcome Secondary: none mentioned
Whalen et al. (50)	27	Prospective observational	Manuscript	Unknown "children"	Severe TBI	Primary: to determine the association between CSF IL-8 levels and patient outcome Secondary: to determine the association between CSF IL-8 in TBI patients and non-TBI controls (n = 24)
Neurophysiologic association						
Muller et al. (51)	25	Prospective observational	Manuscript	41 years (range: unknown)	Severe TBI	Primary: to evaluate the relationship between CSF IL-6, IL-8, and IL-10 with TCD defined CBF Secondary: none mentioned
Stein et al. (52)	14 with CSF cytokines	Prospective observational	Manuscript	31.6 years (range: unknown)	Severe TBI	Primary: to determine the relationship between CSF cytokines with ICP and patient outcome Secondary: none mentioned
Nil association studies						
Amick et al. (53)	24	Prospective observational	Manuscript	5.4 years (range: 0.2–16 years)	Moderate–severe TBI	Primary: to determine the association between IL-2, IL-4, IL-6 and IL-12 with patient outcome Secondary: compare IL levels in CSF to non-TBI controls (n = 12)
Buttram et al. (54)	36	Prospective observational	Manuscript	6.9 years (range: unknown)	Severe TBI	Primary: to measure CSF cytokines and determine the impact of moderate hypothermia on expression. Also determine the link between CSF cytokines and outcome Secondary: compared CSF cytokine profile to non-TBI controls (n = 10)
Csuka et al. (55)	28	Prospective observational	Manuscript	36 years (range: 16–67 years)	Severe TBI	Primary: to determine the association between various CSF and serum cytokines Secondary: to determine the association between CSF cytokines with outcome and ICP

(*Continued*)

TABLE 2 | Continued

Reference	Number of patients	Study type	Article location	Mean age (years)	Patient characteristics	Primary and secondary goal of study
Diamond et al. (56)	59 with CSF cytokines	Prospective observational	Manuscript	Unclear mean age for CSF cytokine cohort	Moderate–severe TBI	Primary: to determine the association between serum and CSF cytokine levels with the development of PTE Secondary: to compare serum and CSF levels with healthy control values. Also assess genetic IL-1b associations with PTE
Goodman et al. (57)	23	Prospective observational	Manuscript	32.7 years (range: 15–57 years)	Severe TBI	Primary: to compare CSF and jugular venous cytokine profiles Secondary: to compare cytokine profiles to ICP and CPP
Gopcevic et al. (58)	20	Prospective observational	Manuscript	53 years (range: unknown)	Severe TBI	Primary: to determine the association between jugular serum and CSF IL-8 levels with in-hospital mortality Secondary: to determine the association between jugular plasm and CSF IL-8 levels
Lenzlinger et al. (59)	41	Prospective observational	Manuscript	38 years (range: 15–74 years)	Severe TBI	Primary: to determine the association between CSF and serum cytokines with patient outcome Secondary: to compared serum and CSF cytokine profiles
Maier et al. (60)	29	Prospective observational	Manuscript	54.8 years (range: 16–85 years)	Severe TBI	Primary: to determine the CSF profile for two soluble tumor necrosis factor receptors (TNFR's) Secondary: to determine the association between CSF sTNFR levels and patient outcome
Maier et al. (61)	29	Prospective observational	Manuscript	45.5 years (range: 18–75 years)	Severe TBI	Primary: to evaluate the correlation between CSF and serum cytokine Secondary: to determine the association between cytokine profile and patient outcome. Also, compare to CSF from healthy volunteers ($n = 35$)
Morganti-Kossmann et al. (62)	42	Unclear	Meeting abstract	Unknown	Severe TBI with various primary and secondary injuries	Primary: to determine the association between serum and CSF cytokines with injury patterns Secondary: to determine the association between cytokine profiles and patient outcome
Newell et al. (63)	66	Retrospective case series	Manuscript	6 years (range: 0.1–16 years)	Severe TBI	Primary: to measure inflammatory markers in the CSF linked to T-cell activation Secondary: to comment on the association between these markers and patient outcome. Also compare levels to healthy controls
Ross et al. (64)	50	Prospective observational	Manuscript	21 years (range: 4–70 years)	Severe TBI	Primary: to compare serum and CSF TNF-a in TBI patients to healthy controls ($n = 46$) Secondary: to compare TNF-a levels to patient outcome
Uzan et al. (65)	11	Prospective observational	Manuscript	28.5 years (range: 2.5–53 years)	Severe TBI	Primary: to determine the association between NO metabolic products and IL-8 Secondary: to determine the association between NO and IL-8 with patient outcome

TBI, traumatic brain injury; sTBI, severe TBI; RCT, randomized control trial; ICP, intracranial pressure; CPP, cerebral perfusion pressure; CSF, cerebrospinal fluid; CBF, cerebral blood flow; $PbtO_2$, partial pressure of oxygen in brain tissue; TCD, transcranial Doppler; DC, decompressive craniectomy; IL, interleukin; a, alpha; b, beta; ra, receptor antagonist; TNF, tumor necrosis factor; NO, nitrous oxide; TNFR, tumor necrosis factor receptor; NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; GDNF, glial-derived neurotrophic factor; PTE, post-traumatic epilepsy; PTD, post-traumatic depression; PCA, principle component analysis.

of sample collection varied as well, with the typical collection period of 5–7 days.

Numerous different panels of cytokines were evaluated within the CMD samples, across the studies included within the review. The most commonly studied cytokines included IL-1b, IL-1ra, IL-6, IL-8, and IL-10. Details of CMD technique and catheter locations are listed in **Table 3**.

CSF Cytokine Review

Sampling of CSF was conducted through external ventricular drains (EVDs) in almost all patients described within the studies included in the CSF cytokine systematic review (30–65). Sampling and analysis frequency varied significantly from study to study with sampling occurring from every 6 h to daily. Duration of sampling varied as well, up to 21 days post-injury (35).

TABLE 3 | CMD cytokine measures and outcomes.

Reference	Catheter location and measured CMD cytokines	Interventional therapies applied during measurement	Primary outcome	Secondary outcome	Complications to CMD	Conclusions
Cederberg et al. (11)	Mixed locations IL-6/IL-8 Unclear sampling interval [3 samples in each patient over course of intensive care unit (ICU) stay] Perfusate not specified	Not specified	6/7 patients survived IL-6 and IL-8 was increased in survivors Peri-lesional location of CMD catheter yielded higher IL-6 and IL-8 levels	N/A	Not specified	IL-6/IL-8 are increase in CDM both in "healthy" and peri-lesional tissue
Figaji et al. (12)	Unclear locations IL-1a, IL-1b, IL1-ra, IL-6, IL-8, and IL-10; VEGF, and MCP-1 Unclear sampling interval Perfusate not specified	Not specified	Variable individual cytokine responses IL-6 and IL-8 were the most consistently elevated across all patients	N/A	Not specified	IL-6/IL-8 are consistently increased in CMD in pediatric sTBI
Guilfoyle et al. (13)	2× CMD catheters per patients (1 healthy tissue, 1 peri-lesional) "42 cytokines" IL-7 and IL-8 Unclear sampling interval Perfusate not specified	Not specified	IL-7 ($p < 0.05$) and IL-8 (<0.05) were found to be higher in peri-lesional tissue IL-1b and interferon gamma (INF-g) were higher in peri-lesional tissue within the first 72 h post-injury	N/A	Not specified	IL-7/IL-8 are higher in peri-lesional tissue IL-1b and INF-g are higher in peri-lesional tissue within the first 72 h
[a]Helmy et al. (14)	Area of "diffuse injury" EGF, Eotaxin, FGF-2, fms-like tyrosine kinase 3 (Flt3) lig, Frac, G-CSF, GM-CSF, GRO, IFN-a2, IFN-g, IL-1a, IL-1b, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, inducible protein (IP)-10, MCP-1, MCP-3, MDC, MIP-1a, MIP-1b, PDGF-AA, PDGF-AAAB, regulated on activation, normal T cell expressed and secreted (RANTES), sCD40L, sIL-2R, TGF-a, TNF q6 h pooled sampling over 5 days 3.5% human ablumin solution perfusate	Not specified	IL-1b and TNF are covariate IL-1ra and IL-1a are covariate MIP-1a and MIP-1b were coexpressed Earlier temporal expression of IL-6, GRO, G-CSF, IP10 compared to IL-10, MCP-3, IL-17	N/A	Not specified	PCA of CMD cytokine profiles yields covariate relationships between specific cytokines and temporal expression pattern
[a]Helmy et al. (15)	Double side-by-side in six patients (to analyze perfusate), and single catheter in six patients—unclear tissue location EGF, Eotaxin, FGF-2, Flt3 lig, Frac, G-CSF, GM-CSF, GRO, IFN-a2, IFN-g, IL-1a, IL-1b, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12p40, IL-12p70, IL-13, IL-15, IL-17, IP-10, MCP-1, MCP-3, MDC, MIP-1a, MIP-1b, PDGF-AA, PDGF-AAAB, RANTES, sCD40L, sIL2R, TGFa, TNF q6 h pooled sampling over 5 days Assessed both crystalloid and 3.5% human albumin perfusate	Unclear; two patients under went DC for refractory ICP	1. Albumin perfusate led to significantly higher fluid recovery compared to crystalloid. Albumin perfusate led to significantly higher cytokine recovery (18 cyotkines) 2. Brain concentrations of 23 cytokines were significantly higher than jugular plasma concentrations (ex. IL-1ra, IL-1a, IL-1b, IL-6, IL-8, IL-10, IL-12p70, MCP-1) Many cytokines displayed a temporal expression, with expression within the first 72 h (e.g., TNF, IL-7, IL-8, MIP1a, sCD40L, IL-1β, GRO, PDGF, AA, RANTES, MIP-1b, IL-1ra, G-CSF, IP10, IL-6)	N/A	Not specified	1. Ablumin CMD perfusate led to increased fluid and cytokine recovery 2. Brain cytokine concentrations were significantly higher than jugular plasma for 23 cytokines. Many cytokines displayed a temporal expression patter with early expression post-injury (72 h)

(*Continued*)

TABLE 3 | Continued

Reference	Catheter location and measured CMD cytokines	Interventional therapies applied during measurement	Primary outcome	Secondary outcome	Complications to CMD	Conclusions
[b]Helmy et al. (16)	Right frontal location (in setting of diffuse injury) 42 cytokine array q6 h pooled sampling for 5 days Isotonic central nervous system perfusate	Group 1 (n = 10): after baseline 6 h CMD samples; received 100 mg rhIL-1ra subcut Repeated q24 h for total of five doses Group 2 (n = 10): control group No specifics on other ICU therapies	1. No complications secondary to rhIL-1ra were seen. 2. CMD IL-1ra concentrations were significantly higher in the treatment group vs. control (p = 0.02), with variation over time (p < 0.0001) 3. MDC was significantly lower in the rhIL-1ra (p = 0.05)	N/A	Not specified	1. rhIL-1ra appears safe in severe diffuse TBI 2. rhIL-1ra reaches the brain extracellular fluid 3. MDC was lower in the rhIL-1ra group
[b]Helmy et al. (17)	Right frontal location (in setting of diffuse injury) 42 cytokine array q6 h pooled sampling for 5 days 3.5% human albumin perfusate	Group 1 (n = 10): after baseline 6 h CMD samples; received 100 mg rhIL-1ra subcut Repeated q24 h for total of five doses Group 2 (n = 10): control group No specifics on other ICU therapies	Based on PCA it was found that cytokines associated with macrophage recruitment were decreased in the rhIL-1ra group (MIP-1a, MCP-3, Fractalkine, GM-CSF)	N/A	Not specified	CMD macrophage base cytokines are decreased in rhIL-1ra-treated patients
Hillman et al. (18)	Paired CMD catheter placement in peri-lesional tissue IL-6 q6 h pooled analysis Ringer's/dextran 60 or human albumin perfusate	Not specified	CMD IL-6 concentrations varied depending on underlying condition and secondary injury (i.e., ischemia) The temporal expression of CMD measured IL-6 varied between patients	N/A	1 catheter membrane failure	CMD IL-6 concentrations varied from patient to patient and depending on initial and secondary injury patterns
Hillman et al. (19)	Peri-lesional tissue IL-1b, IL-6 q6 h pooled analysis 3.5% human albumin perfusate	Not specified	CMD biochemical evidence of ischemia (LPR > 30 and glutamate >80 μmol/L for 24 h period) was associated with significant IL-6 increase (p < 0.01), which subsided after ~90 h post-injury (p < 0.001) In those patients without biochemical ischemia, IL-6 levels spiked in the first 48 h (p < 0.01) IL-1b activation was less commonly observed (only 53% of measures)	N/A	Not specified	CMD IL-6 displays a correlation with CMD biochemical ischemia and a temporal correlation post-injury (in the absence of biochemical ischemia)

(*Continued*)

TABLE 3 | Continued

Reference	Catheter location and measured CMD cytokines	Interventional therapies applied during measurement	Primary outcome	Secondary outcome	Complications to CMD	Conclusions
Hutchinson et al. (20)	Unclear tissue location ("frontal cortex") IL-1a, IL-1b, IL-1ra q6 h pooled samples (mean no. samples = 9.1; range = 4–23) Isotonic central nervous system perfusate	Not specified	IL-1a and IL-1b concentrations were lower than IL-1ra A positive correlation between IL-1ra and IL-1b was seen ($p = 0.028$) No correlation between IL-1b and IL-1ra was found No correlation between cytokines and CMD glucose, glutamate, LPR	ICP: ICP was negatively correlation to IL-1ra ($p = 0.041$) No correlation between other cytokines and ICP No correlation between cytokines and CPP Outcome: significant relationship between mean IL-1ra levels and poor outcome (dichotomized GOS at 6 months) ($p = 0.018$)—high IL-1ra was associated with good outcome No relationship between IL-1a and IL-1b with outcome	Not specified	1. The appears to be a correlation between IL-1ra and IL-1b 2. There is a negative correlation between ICP and IL-1ra 3. Mean IL-1ra levels correlate to patient outcome at 6 months
Mellergard et al. (21)	Mixed locations; some patients with two catheters (unclear which patients) IL-1b, IL-6, IL-8, FGF-2, MIP-1b, RANTES, VEGF, IL-10 q6 h pooled samples for 36 h Ringer-dextran 60 perfusate	Not specified	IL-1b peaked in the first 12 h period IL-6 peaked after 12 h post-insertion IL-8 peaked within the first 6 h post-insertion MIP-1b peaked within the first 6 h post-insertion FGF-2 peaked within the first 6 h post-insertion IL-10, VEGF, and RANTES did not show a temporal profile	N/A	Not specified	CMD catheter insertion leads to IL-1b/IL-6/IL-8/MIP1b within the first 6–12 h, which then decrease during the subsequent time afterward

(*Continued*)

TABLE 3 | Continued

Reference	Catheter location and measured CMD cytokines	Interventional therapies applied during measurement	Primary outcome	Secondary outcome	Complications to CMD	Conclusions
[c]Mellergard et al. (22)	Paired catheters (1 peri-lesonal; 1 healthy tissue)—used the catheter with highest glycerol levels for measuring cytokines IL-1b, IL-6, IL-10 q6 h pooled analysis for 7 days Ringer-dextran 60 perfusate	Not specified; various surgical procedure for hemotomas in TBI group	IL-1b increased during the first 48 h, and then decreased IL-6 increased over the first 48 h, and then decreased IL-10 remained elevated throughout the measurement period	N/A	Not specified	IL-1b and IL-6 display a peak elevation during the first 48 h post-TBI IL-10 remains elevated through the first 7 days post-TBI
[c]Mellergard et al. (23)	Paired catheters (1 peri-lesonal; 1 healthy tissue)—used the catheter with highest glycerol levels for measuring cytokines FGF-2, VEGF q6 h pooled analysis for 7 days Ringer-dextran 60 perfusate	Not specified; various surgical procedure for hemotomas in TBI group	FGF-2 levels peaked at day 3 post-TBI VEGF levels peaked on day 2 post-TBI	N/A	Not specified	FGF-2/VEGF levels peaked on days 3 and 2 post-TBI
Mellergard et al. (24)	Unclear location IL-1b, IL-6, IL-8, FGF-2, MIP-1b, RANTES, VEGF, IL-10 q6 h pooled sample analysis Ringer-dextran 60 perfusate	Local protocols; not otherwise specified	IL-1b, IL-8, and IL-10 did not display age-related differences VEGF, MIP-1b, and RANTES were different in the <25 years age group vs. over 25 years age FGF-2 levels were significantly higher in the >65-year-old group ($p < 0.0001$)	N/A	Not specified	There may be an age-related difference in the expression of VEGF, MIP-1b, RANTES, and FGF-2 post-TBI
Mondello et al. (25)	Unclear location IL-1b, IL-6, TNF-a, INF-g Unclear sampling interval Unclear perfusate	Not specified	IL-6 showed high initial values that then decreased, in contrast IL-1beta, TNF-alpha and INF-gamma showed later elevations UCH-L1 levels negatively correlated ($p < 0.05$) with IL-1beta, widely used biomarker of inflammation	N/A	Not specified	Variable cytokine temporal profiles are seen post-TBI
Parez-Barcena et al. (26)	Right frontal location; unclear tissue quality IL-1b, IL-6, IL-8, IL10, IL-12, TNF-a q8 h sample analysis (up to 248 h duration) Isotonic central nervous system perfusate	Varied ICP/CPP directed therapies; some use of barbiturates	IL-1b, IL-6, and IL-8 peaked during first 24 h post-injury IL-10 remained unchanged during the sampling period	ICP: no correlation between IL-1b, IL-6, IL-8 and IL-10 with ICP $PbtO_2$: no clear correlation between cytokines and $PbtO_2$	Not specified	1. IL-1b, IL-6 and IL-8 peaked within the first 24 h post-injury 2. No clear association was found between cytokines and ICP, $PbtO_2$, CT changes

(*Continued*)

TABLE 3 | Continued

Reference	Catheter location and measured CMD cytokines	Interventional therapies applied during measurement	Primary outcome	Secondary outcome	Complications to CMD	Conclusions
				CT: no association found between cytokines and subsequent CT defined swelling or lesion change		
Roberts et al. (27)	Healthy tissue IL-1a, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, and TNF-a q6 h pooled analysis (up to 156 h of monitoring) Isotonic central nervous system perfusate	Varied; one patient had DC	IL-1a, IL-1b, and TNF-a were elevated initially after injury IL-6 and IL-8 were substantially higher in the CMD compared to other cytokines IL-5 was barely detectable Similar cytokine concentrations were seen in CSF and CMD, which were both substantially higher than jugular plasma sampled Increase CMD concentrations of MMP-8 and MMP-9 were seen with increases in the levels of IL-1a, IL-2, and IL-1a and -2 and TNF-a, respectively. In contrast, the CMD levels of MMP-7 decreased with increases in IL-1b, IL-2, and IL-6	Neuro Exam: IL-1b, IL-4 and TNF-a levels were substantially higher in those with loss of pupillary reactivity ICP: IL-2 displayed a negative correlation to ICP TNF-a displayed a negative correlation to ICP CPP: IL-6 and IL-8 displayed a negative correlation to CPP $PbtO_2$: no correlation found between cytokines and $PbtO_2$ Outcome: no correlation between cytokines and GOS	Not specified	1. IL-1a, IL-1b, TNF-a, IL-6 and IL-8 predominate the cytokine response post TBI 2. Various patterns of MMP changes are seen in correlation with changes in cytokine expression 3. IL-1b, IL-4 and TNF-a levels were higher in those with loss of pupillary reactivity 4. IL-6 and IL-8 correlation with CPP. TNF-a correlations with ICP

(*Continued*)

TABLE 3 | Continued

Reference	Catheter location and measured CMD cytokines	Interventional therapies applied during measurement	Primary outcome	Secondary outcome	Complications to CMD	Conclusions
Winter et al. (28)	Peri-lesional IL-1b, IL-6, NGF q3 h sampling (for 6 days) Normal saline perfusate	Not specified	CMD cytokine analysis is feasible and safe	Peak cytokine levels were seen within the first 36 h post-injury IL-1b predominated with substantially higher concentrations compared to IL-6 and NGF IL-6 was high in survivors, while NGF was lower in non-survivors	None	1. CMD cytokine analysis is feasible 2. IL-1b may be the predominant CMD cytokine expressed 3. Unclear patterns in survivors vs. non-survivors
Winter et al. (29)	Healthy tissue IL-1b, IL-6, NGF q3 h sampling Normal saline perfusate	Not specified	Higher IL-6 was seen in survivors ($p = 0.04$) Peak IL-6 correlated to GOS at 6 months ($p = 0.03$) Peak NGF:IL-1b ratios were significantly lower in survivors ($p = 0.01$)	N/A	None	IL-6 levels in CMD samples may correlation to survival and GOS at 6 months

TBI, traumatic brain injury; sTBI, severe TBI; GOS, Glasgow outcome scale; CMD, cerebral microdialysis; RCT, randomized control trial; ICP, intracranial pressure; CT, computed tomography; $PbtO_2$, brain tissue oxygen monitoring; CPP, cerebral perfusion pressure; CSF, cerebrospinal fluid; LPR, lactate:pyruvate ratio; DC, decompressive craniectomy; μmol, micromolar; mm Hg, millimeters of mercury; L, liter; μmol, micromolar; IL, interleukin; a, alpha; b, beta; g, gamma; TNF, tumor necrosis factor; INF, interferon; MCP, monocyte chemoattractant protein; MIPs, macrophage inflammatory proteins; TGF, transforming growth factor; NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; GDNF, glial-derived neurotrophic factor; TNFR, tumor necrosis factor receptor; GM-CSF, granulocyte macrophage colony stimulating factor; sVCAM, soluble vascular cell adhesion molecule; sICAM, soluble intracellular adhesion molecule; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; MDC, macrophage-derived chemokine; FGFR, fibroblast growth factor receptor.

[a]*Same patient population reported in both Helmy et al. (14) and Helmy et al. (15).*

[b]*Same patient population described in Helmy et al. (16) and Helmy et al. (17).*

[c]*Same patient population reported in both Mellergard et al. (22) and Mellergard et al. (23).*

TABLE 4 | CSF cytokine measures and outcomes.

Reference	Interval of cytokine measure	Measured CMD cytokines	Interventional therapies applied during measurement	Outcome(s) of interest (patient outcome, neurophysiologic outcome, tissue outcome)	Other outcomes	Conclusions
Abboud et al. (30)	q12-h Intervals for 5 days	IL-1α,IL-1β, IL-2, IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, MIP-1a, MIP-1b, TNF-a, VEGF	Not specified	GOS at 6 and 12 months post-injury Statistically significant differences in IL-4, IL-5, IL-6, IL-8, IL-13, and TNF-α (all $p < 0.05$) were observed between TBI survivors vs. non-survivors over 5 days	N/A	Elevated IL-4, IL-6, IL-8, IL-23, and TNF-a levels may be associated with poor outcome at 6 and 12 months Similarly, low IL-5 and IL-13 may be associated with poor outcome
Bell et al. (31)	q24-h Intervals for 3 days Control group had banked CSF	IL-6, IL-10	High variable; barbiturates and various ICP/CPP-directed therapies	Mortality (at unclear interval) IL-6 is not associated with mortality IL-10 is associated with mortality ($p = 0.022$)	IL-6 and IL-10 levels were increased compared to controls	Elevated IL-10 levels may be associated with mortality
Chiaretti et al. (32)	At 2 and 48 h post-injury Controls were initially investigated for meningitis but were found to be negative. CSF was banked	IL-6, NGF	Highly protocolized therapy; seemingly homogenous between patients	GOS at 6 months Low IL-6 and NGF at 2 h post-injury was associated with good outcome ($p < 0.01$) Increased IL-6 variation between the two time points was correlated with better outcome	IL-6 and NGF were both elevated and increased between the two sampling periods IL-6 and NGF were positively correlated at both time periods	Lower IL-6 and NGF levels early post-TBI may be associated with better outcome at 6 months
Chiaretti et al. (33)	At 2 and 48 h post-injury	IL-1b, IL-6, NGF, BDNF, GDNF	Highly protocolized therapy; seemingly homogenous between patients	GOS at 6 months Low NGF at 2 h ($p < 0.01$) and high NGF/IL-6 ($p = 0.02/p < 0.01$) at 48 h were associated with better outcome Low IL-1b at 48 h was associated with better outcome ($p < 0.01$)	N/A	Low initial NGF, followed by increased NGF/IL-6 may be associated with good outcome at 6 months Low IL-1b at 48 h may be associated with better outcome at 6 months
Chiaretti et al. (34)	At 2 and 24 h post-injury	IL-1b and IL-6	Highly protocolized therapy; seemingly homogenous between patients	Dichotomized GOS at 6 months (good = 4 or 5; poor = 3 or less) Higher CSF IL-1b and IL-6 at both 2 h and 24 h were seen in those patients with poor outcome at 6 months	IL-1b and IL-6 at 2 h were higher in the TBI cohort	Elevated IL-1b and IL-6 at both 2 and 24 h post-injury may be associate with poor outcome at 6 months
Hans et al. (35)	Daily CSF samples up to 21 days post-injury	IL-6 and sIL-6R	Not specified	Dichotomized GOS at 6 months (good = 4 or 5; poor = 3 or less) High IL6/sIL-6R was associated with poor outcome at 6 months	CSF levels of IL-6 and sIL-6R were higher than compared to plasma	Elevated IL-6/sIL-6R may be associated with poor outcome at 6 months
Hayakata et al. (36)	6, 12, 24, 48, 72, and 96 h after injury	TNF-a, IL-1, IL-6, IL-8, and IL-10	Varied therapies; hypothermia and other ICP/CPP-directed approaches	Dichotomized GOS at 6 months (good = 4 or 5; poor = 3 or less) CSF IL-1b was found to be higher in those with poor outcome	ICP: IL-1b was significantly positively correlated with ICP throughout the entire study ($p < 0.05$)	1. Elevated IL-1b may be associated with poor outcome at 6 months 2. Elevated IL-1b may be associated with elevated ICP

(*Continued*)

TABLE 4 | Continued

Reference	Interval of cytokine measure	Measured CMD cytokines	Interventional therapies applied during measurement	Outcome(s) of interest (patient outcome, neurophysiologic outcome, tissue outcome)	Other outcomes	Conclusions
					Various cytokine elevations were seen during S100B elevations. IL-1b peaks was correlated with S100B peak ($p < 0.005$)	
Jamil et al. (37)	Unclear interval; "acute" period post-TBI	IL-1b, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, TNF-a, sICAM-1, sVCAM-1, sFAS	Not specified	Patient health questionnaire (PHQ-9) at 6 and 12 months post-injury Acute CSF IL-6 ($p = 0.008$), IL-8 ($p = 0.034$), and ICAM1 ($p = 0.025$) levels were higher among patients who would go on to develop depression 6 months after injury Acute CSF TNF-a ($p = 0.036$), IL-4 ($p = 0.007$), and IL-1b ($p = 0.001$) levels were individually associated with lower depression risk at 12 months post-injury	N/A	1. Elevated IL-6 and IL-8 may be associate with depression at 6 2. TNF-a, IL-4, and IL-1b may be associated with lower chance of depression at 12 months
Juengst et al. (38)	Within first week of injury	IL-4, IL-5, IL-8, IL-12, TNF-a, sVCAM, sICAM	Not specified	Apathy subscale of the frontal systems behavior scale, collected at 6 and 12 months post-TBI Higher acute CSF IL5, sVCAM, and sICAM with apathy at 6 months and lower acute serum TNFalpha, IL8, and IL5 with apathy at 12 months ($p < 0.05$)	N/A	Higher acute CSF IL5, sVCAM, and sICAM with apathy at 6 months and lower acute serum TNFalpha, IL8, and IL5 with apathy at 12 months
Juengst et al. (39)	2 times daily for 6 days	TNF-a	Not specified	At 6 and 12 months post-injury, FrSBe disinhibition subscale; suicidal endorsement was assessed by the PHQ-9 No relationship between TNF-a in CSF and suicidality at 6 or 12 months Acute serum TNFa levels were inversely associated with 12-month disinhibition ($r = 0.520$, $p = 0.027$) and achieved borderline significance with 6-month disinhibition ($r = 0.470$, $p = 0.057$)	TBI patients had significantly higher CSF TNF-a levels compared to controls	Acute levels of TNF-a may correlate to 6 and 12 month rates of disinhibition
Juengst et al. (40)	2 times daily up to 6 days	IL-1β, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, TNF-a, sVCAM-1, sICAM-1, sFAS	Not specified	PHQ-9 was administered to participants at 6 and 12 months after injury The inflammatory cell surface markers sVCAM-1, sICAM-1, and sFAS in the CSF were each positively associated with PTD at 6 months ($p < 0.02$ for all comparisons). The cytokine IL-8 was positively associated with PTD at 12 months ($p < 0.02$), while the cytokine IL-7 was inversely associated with PTD at 12 months ($p < 0.05$)	IL-1β, IL-4, IL-6, IL-7, IL-8, IL-10, TNF-α, sVCAM-1, sICAM-1, and sFAS ($p < 0.05$) were significantly elevated compared to controls	1. Elevated sVCAM-1, sICAM-1 and sFAS may be associated with PTD at 6 months 2. Elevated IL-7 and IL-8 may be associated with PTD at 12 months
Kirchhoff et al. (41)	Upon EVD insertion, then at 12, 24, and 48 h post-injury	IL-10	Not specified	Mortality at unspecified interval IL-10 was significantly higher in non-survivors	IL-10 was higher at all time points compared to non-TBI controls	Elevated CSF IL-10 at admission was associated with mortality

(*Continued*)

TABLE 4 | Continued

Reference	Interval of cytokine measure	Measured CMD cytokines	Interventional therapies applied during measurement	Outcome(s) of interest (patient outcome, neurophysiologic outcome, tissue outcome)	Other outcomes	Conclusions
	Control group: CSF gained from spinal anesthetics in elective non-TBI surgical cases					
Kossmann et al. (42)	q24 h for unclear duration Control group: non-TBI patients (1 VPS and 2 Dx LP)	IL1b, IL-6, TNF-a, NGF	Various therapies; heterogeneous across population	Dichotomized GOS at 3 months (good = 4 or 5; poor = 3 or less) High IL-6 levels were associated with NGF presence in CSF NGF levels were elevated in those with better outcomes	IL-6 and NGF were high in TBI patients compared to control samples	NGF may be elevated in those with good outcome
Kumar et al. (43)	2 times daily for 5 days	IL-6	Not specified	Dichotomized GOS at 6 and 12 months (good = 4 or 5; poor = 3 or less) Association between high IL-6 upon admission and 6-month GOS ($p = 0.003$)	IL-6 levels were higher in TBI compared to controls	High IL-6 during the first 5 days of injury may be associated with poor outcome at 6 months
Kumar et al. (44)	2 times daily for up to 5 days	IL-1β, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, TNF-a, sVCAM-1, sICAM-1, sFAS	Not specified	Trichotomized GOS at 6 and 12 months (good = 4 or 5; poor = 3 or 2; dead = 1) Individuals in cluster 1 (increased sICAM-1, sFAS, IL-10, IL-6, sVCAM-1, IL-5, and IL-8) had a 10.9 times increased likelihood of GOS scores of 2/3 vs. 4/5 at 6 months compared to cluster 2 (increased IL-12, IL7, IL-4)	Cytokines were elevated in TBI patients compared to controls	Elevated IL-5, IL-6, IL-8, IL-10, sVCAM-1, and sICMA-1 may be associated with poor outcome at 6 months
Kushi et al. (45)	Admission, 24, 72, and 168 h post-injury	IL-6, IL-8	High protocolized treatment; fairly homogeneous therapy	Mortality at unspecified interval IL-6 and IL-8 levels were significantly higher in CSF compared to serum IL-6 and IL-8 levels were significantly higher in non-survivors	N/A	Elevated IL-6 and IL-8 during the first week post-TBI may be associated with mortality
Nwachuku et al. (46)	q6 h for 5 days	IL-1b, IL-6, TNF-a, IFN-a, IL-12p70, IL-10, and IL-8	Not specified	Dichotomized GOS at 3, 6, 12, and 24 months (good = 4 or 5; poor = 3 or less) Mean 5-day levels of IFN-a, IL-10, IL-12 p70, IL-1, IL-6, IL-8, and TNF-a were associated with outcome ($p < 0.05$)	N/A	Elevated mean 5-day levels of various cytokines may be associated with poor outcome at 3, 6, 12, and 24 months post-injury
Santarseiri et al. (47)	2 times daily for up to 6 days	IL-1β, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12, TNF-a, sVCAM-1, sICAM-1, sFAS	Not specified	Dichotomized GOS at 6 months (good = 4 or 5; poor = 3 or less) Cortisol: high cortisol patients were more likely to have elevated IL-10, IL-1b, IL-6, sFas, sICAM-1, sVCAM-1 and TNFa ($p < 0.01$ all comparisons, except IL-1b, $p < 0.05$) compared to low cortisol patients Outcome: significant associations between GOS and mean levels of IL-10, IL-6, IL-8, sFas, sICAM-1 ($p < 0.01$) and TNF-a ($p < 0.05$), with lower levels associated with favorable outcome	N/A	Low mean IL-6, IL-8, IL-10, sICAM-1, and TNF-a may be associated with good outcome at 6 months post-injury

(*Continued*)

TABLE 4 | Continued

Reference	Interval of cytokine measure	Measured CMD cytokines	Interventional therapies applied during measurement	Outcome(s) of interest (patient outcome, neurophysiologic outcome, tissue outcome)	Other outcomes	Conclusions
Shiozaki et al. (48)	q6 h for unclear duration	IL-1b, IL-1ra, IL10, TNF-a, sTNFr-I	Highly Protocolized therapy	Dichotomized GOS at 6 months (good = 4 or 5; poor = 3 or less) IL-1b, IL-1ra, sTNFr-I, and IL-10 were significantly higher in patients with an unfavorable outcome than in patients with a favorable outcome ($p = 0.006$, $p = 0.009$, $p = 0.003$, and $p = 0.012$, respectively)	IL-1b, IL-1ra, sTNFr-I, and IL-10 were significantly higher in patients with high ICP than those with low ICP ($p = 0.002$, $p = 0.006$, $p = 0.009$, and $p = 0.009$, respectively). However, the CSF concentrations of TNF-a did not differ between patients with high ICP and those with low ICP	1. Elevated IL-lb, IL-1ra, IL-10, and sTNFr-I may be associated with poor outcome at 6 months 2. Elevated IL-1b, IL-1ra, IL-10, sTNFr-I may be associated with high ICP
Singhal et al. (49)	Unclear interval	IL-1b, IL-6	Not specified	*SSEP: positive* correlation between IL-6 and SSEP96 (mean change in SSEP over 96 h) ($p = 0.0133$) Outcome: GOS at 3 months Peak IL-6 levels were associated with good outcome ($p = 0.026$)	N/A	1. Elevated IL-6 may be positively correlated to SSEP over the first 96 h 2. Peak IL-6 levels may be associated with outcome at 3 months
Whalen et al. (50)	Unclear sampling intervals	IL-8	Not specified	Mortality at unspecified interval Elevated CSF IL-8 levels were associated with mortality ($p = 0.01$)	IL-8 levels were elevated compared to controls	Elevated IL-8 levels during the first week of injury may be associated with mortality
Neurophysiologic association						
Muller et al. (51)	Daily for 7 days	IL-6, IL-8, IL-10	Not specified	Transcranial doppler (TCD)-defined cerebral blood flow velocity Mean IL-6 and IL-8 level were significantly correlated to MCBFV ($r = -0.341$ and -0.361, respectively; $p < 0.05$)	N/A	Elevated IL-6 and IL-8 in the first 7 days may be negatively correlated to TCD defined MCBFV
Stein et al. (52)	2 times daily for 7 days	IL-1b, IL-6, IL-8, IL-10, and TNF-a	High protocolized therapy	ICP: negative association between early (within first 12 h of injury) IL-6 and ICP ($p = 0.004$) Positive correlation between time spent with CPP below 60 mm Hg and IL-8 levels ($p = 0.001$) Outcome: dichotomized GOSE at 6 months (good = 5–8; poor = 1–4) No association between CSF cytokines and outcome	N/A	1. Elevated IL-6 within the first 12 h of injury may be associated with low ICP 2. Elevated IL-8 levels may be associated with low CPP
Nil association studies						
Amick et al. (53)	Unclear time frame post-TBI (from 4 to 122 h after injury)	IL-2, IL-4, IL-6, IL-12	Highly variable; barbiturates and various ICP/CPP-directed therapies	GOS at 6 months post-Injury	IL-6 and IL-12 were increased compared to control group	No association between IL-2, IL-4, IL-6, and IL-12 with GOS at 6 months

(*Continued*)

TABLE 4 | Continued

Reference	Interval of cytokine measure	Measured CMD cytokines	Interventional therapies applied during measurement	Outcome(s) of interest (patient outcome, neurophysiologic outcome, tissue outcome)	Other outcomes	Conclusions
	Banked samples from a non-TBI control group (n = 12); CSF gained from investigations for meningitis			No correlation between measured CSF cytokines and GOS		
Buttram et al. (54)	Collected 18, 24, 48, and 72 h post-injury	IL-1a, IL-1b, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12p70, IL-13, IL-15, IL-17, IP-10, eotaxin, TNF-a, INF-g, MCP-1, MIP-1a	Not well specified; half the groups was subjected to moderate hypothermia for 48 h (32–33°C)	Dichotomized GOS at 6 months No association between CSF cytokines and outcome	Cytokine levels in TBI patients were significantly higher compared to controls	There is no association between CSF cytokines and outcome at 6 months
Csuka et al. (55)	Daily until EVD removal	IL-6, IL-10, TNF-a, TGF-B1	Unclear ICP/CPP-directed therapies	Outcome: GOS at 3–6 months No correlation found between cytokines and outcome ICP: no correlation between cytokines and ICP	IL-10 was found in both CSF and serum during the measurement period	CSF cytokines do not correlate to outcome at 3–6 months CSF cytokines do not correlate to ICP
Diamond et al. (56)	q12 h for 6 days	IL1b	Not specified	EEG and Epileptologist defined PTE CSF IL-1b was not statistically associated with PTE	Serum IL-1b levels was associated with PTE	CSF IL-1b levels within the first week of injury is not associated with PTE
Goodman et al. (57)	Unclear sampling interval	IL-1, IL-6, IL-8, IL-10, IL-12, TNF	Not specified	ICP/CPP: no correlation between CSF cytokines and ICP or CPP	Serum and CSF IL-6 and IL-8 were both elevated consistently	CSF cytokines are not associated with changes in ICP and CPP
Gopcevic et al. (58)	Unclear sampling interval	IL-8	Not specified	30-day in-hospital mortality: no correlation between CSF IL-8 levels and patient outcome	No correlation between plasma and CSF IL-8 levels	CSF IL-8 is not associated with in-hospital mortality at 1 month
Lenzlinger et al. (59)	Daily for unclear duration	sIL-2R, B2M, neopterin	Unclear ICP direct therapy	GOS at 4–6 months No association between measured cytokines and outcome	Neopterin levels were higher in CSF than serum B2M and sIL-2R levels were higher in serum	sIL-2R, B2M, and neopterin in CSF have no correlation to outcome at 4–6 months
Maier et al. (60)	Admission and daily up to day 10	sTNFRp55, sTNFRp75	Not specified	GOS at 6 months No correlation between sTNFRp55 or sTNFRp75 and outcome	sTNRFp55 and STNFRp75 is elevated in CSF compared to control	sTNFRp55 and sTNFRp75 CSF levels are not associated with outcome at 6 months
Maier et al. (61)	Admission and daily up to day 14	IL-6, IL-8, IL-10	Not specified	Mortality at unspecified interval No correlation between CSF cytokines and patient outcome	IL-6 and IL-8 were directly correlated with each other with CSF level higher than serum All measured cytokines were higher in TBI patients compared to controls	CSF IL-6, IL-8, and IL-10 levels do not correlated with mortality

(*Continued*)

TABLE 4 | Continued

Reference	Interval of cytokine measure	Measured CMD cytokines	Interventional therapies applied during measurement	Outcome(s) of interest (patient outcome, neurophysiologic outcome, tissue outcome)	Other outcomes	Conclusions
Morganti-Kossmann et al. (62)	Unclear sampling interval	IL-2, IL-4, IL-6, IL-10, TNF, IFN, GM-CSF	Not specified	Unclear outcome scale at unspecified interval No clear association between CSF cytokines and outcome	IL-6 is higher in focal injury patterns	CSF cytokines are not associated with patient outcome
Newell et al. (63)	q12–24 h for 7 days	sIL-2Ra	Not specified	Dichotomized GOS at 6 months (good = 4 or 5; poor = 3 or less) No association between sIL-2Ra and outcome	sIL-2ra levels during the measurement period were no different between TBI and controls	sIL-2Ra isn't significantly elevated post-TBI and does not correlate with outcome
Ross et al. (64)	Unclear sampling interval	TNF-a	Not specified	GOS at 6 months No correlation between TNF-a and outcome	TNF-a in CSF and serum were both elevated	CSF TNF-a displayed no association with patient outcome at 6 months
Uzan et al. (65)	At 6–10, 20–28, 40–56, and 64–74 h post-injury	IL-8	Unclear ICP/CPP-directed therapies	GOS at unspecified interval No correlation between CSF IL-8 and patient outcome	N/A	CSF IL-8 level within the first 2–3 days are not associated with outcome

TBI, traumatic brain injury; sTBI, severe TBI; GOS, Glasgow outcome scale; CMD, cerebral microdialysis; RCT, randomized control trial; ICP, intracranial pressure; CT, computed tomography; $PbtO_2$, brain tissue oxygen monitoring; CPP, cerebral perfusion pressure; CSF, cerebrospinal fluid; LPR, lactate:pyruvate ratio; DC, decompressive craniectomy; µmol, micromolar; mm Hg, millimeters of mercury; L, liter; µmol, micromolar; IL, interleukin; a, alpha; b, beta; g, gamma; TNF, tumor necrosis factor; INF, interferon; MCP, monocyte chemoattractant protein; MIPs, macrophage inflammatory proteins; TGF, transforming growth factor; NGF, nerve growth factor; BDNF, brain-derived neurotrophic factor; GDNF, glial derived neurotrophic factor; TNFR, tumor necrosis factor receptor; GM-CSF, granulocyte macrophage colony stimulating factor; sVCAM, soluble vascular cell adhesion molecule; sICAM, soluble intracellular adhesion molecule; PDGF, platelet-derived growth factor; VEGF, vascular endothelial growth factor; MDC, macrophage derived chemokine; FGFR, fibroblast growth factor receptor.

Like the CMD cytokine papers, the CSF cytokine papers included in this review reported the measurement of various cytokines. The most commonly measured cytokines in CSF reported were IL-1b, IL-1ra, IL-6, IL-8, IL-10, and TNF. The details of CSF sampling and specific cytokines measured can be found in **Table 4**.

Outcomes

CMD Cytokine Review

Given that the CMD cytokine portion of this review was a scoping review of all the literature on CMD cytokines in sTBI, the goals and outcomes reported by the studies were heterogenous, and are listed in **Table 3**.

Only one study described an intervention during the assessment of CMD cytokines. This study was a prospective RCT describing the application of subcutaneous rhIL-1ra post severe diffuse TBI (16). The results described both elevated CMD IL-1ra levels and a reduction in MDC in the IL-1ra treated group. The follow-up retrospective statistical analysis of all CMD measured cytokines described a trend toward an increase in M1-microglia related cytokine activation following administration of rhIL-1ra (17).

Three studies reported the correlation between CMD cytokines and patient outcome (11, 20, 29). Two studies reported a positive association between elevated CMD IL-6 and improved survival, with one describing improved Glasgow Outcome Scale (GOS) at 6 months ($p = 0.03$). One study reported the negative correlation between CMD IL-1ra and poor GOS at 6 months ($p = 0.018$).

Most studies reported the CMD cytokine profile post-TBI and temporal fluctuations (12, 14, 15, 21, 24, 26, 27). Given the myriad of cytokines measured across the studies, it is impossible to describe all of the relationships. Highlighted details can be found in **Table 3**. The main findings included elevated IL-1b, IL-6, and IL-8 within the first 48–72 h post-injury, with these cytokines also displaying peaks during these times (21–23). The CMD IL-10 levels were found to be more uniformly elevated during the sampling periods (22, 26). Finally, some coexpression relationships were found between IL-1b with TNF, IL-1ra with IL-1a, and MIP-1a with MIP-1b (14).

Two studies evaluated the CMD cytokine profile associated with secondary events while in the ICU (18, 19). CMD IL-6 levels were positively associated with episodes of ischemia/metabolic stress, as defined by a lactate:pyruvate ratio greater than 30 and glutamate levels greater than 80 µmol/L.

The relationship of catheter location to CMD cytokine levels was discussed in a couple of papers, with peri-lesional tissue displaying higher cytokine expressions than distant or healthy tissue locations (11, 13). Evaluation of catheter technology (18) and cytokine measure feasibility (28) were also described in a few studies.

CSF Cytokine Review

The 36 papers included in the CSF systematic review (30–65) included both manuscripts, which reported positive associations between CSF cytokine levels and neurophysiologic or patient outcome (30–52), and studies reporting no association (53–65)

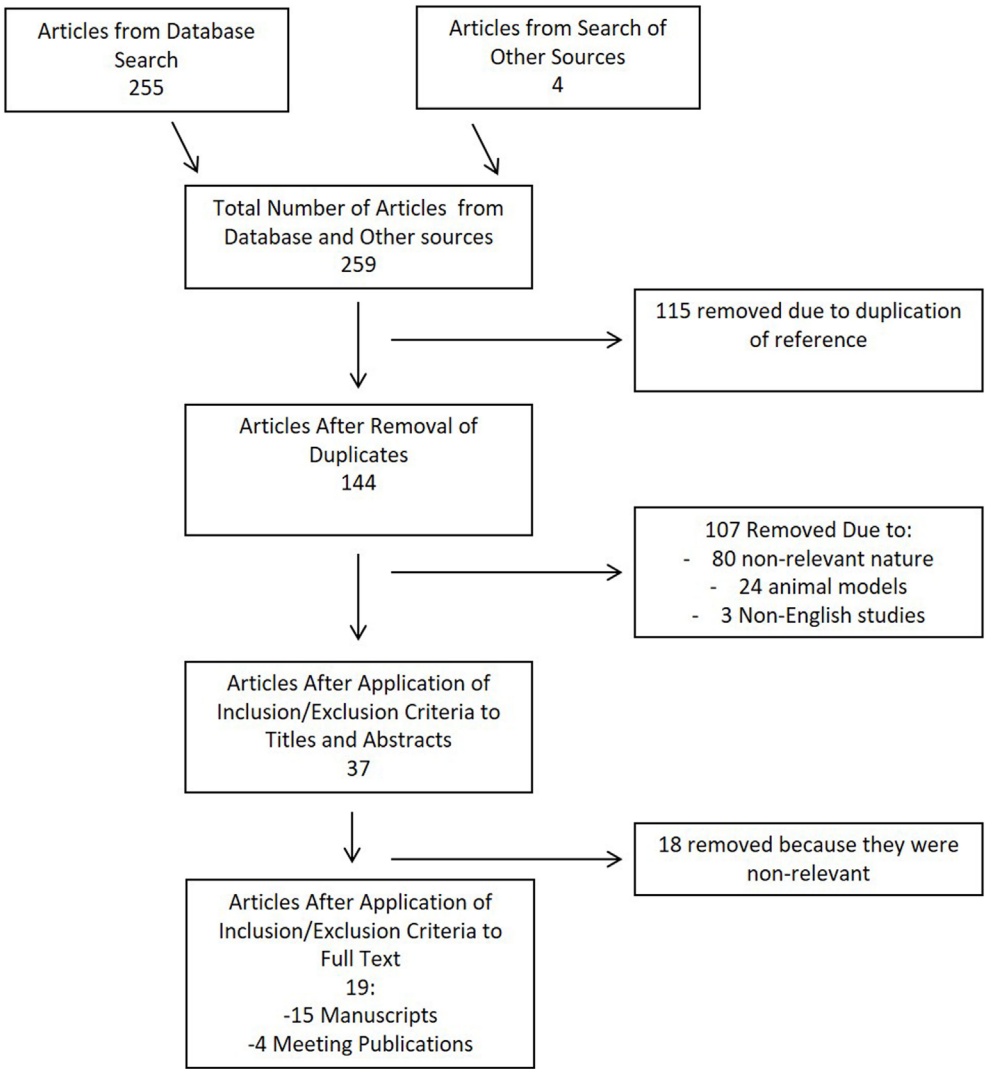

FIGURE 1 | Flow diagram of search results for cerebral microdialysis review.

(i.e., "nil association") between CSF cytokines and the outcomes of interest for the CSF cytokine systematic review. No studies reported an association, "nil" or otherwise, between CSF cytokine measures and tissue outcome as assessed by follow-up neuroimaging. The subsections below describe more details of these outcomes of interest, with further information found in **Table 4**.

Positive Association Studies

Twenty-three papers included within the CSF cytokine review found associations between cytokine levels and both neurophysiologic and patient outcomes. Twenty-one described the association between CSF cytokines and patient outcome (30–50). Five papers discussed the association between CSF cytokine measures and neurophysiologic outcomes (36, 48, 49, 51, 52).

Patient Outcome. Cerebrospinal fluid levels of several cytokines were related to functional patient outcomes. The most common outcomes specified were: overall mortality or GOS at 6–12 months post-injury. The strongest relationships between cytokines and patient outcome were for IL-1b, IL-1ra, IL-6, IL-8, IL-10, and TNF.

A strong positive correlation between CSF measured IL-6 and IL-8 with poor GOS was the most commonly described relation between CSF cytokines and patient outcome (30, 32, 35, 36, 42–47, 49, 50). Similarly, a strong association between elevated CSF measured IL-10 and poor patient outcome was described in five studies (31, 41, 46–48). Elevated CSF IL-1b was found to be associated with mortality and worse GOS at 6 months in four studies (33, 34, 36, 49). Finally, CSF TNF-alpha (TNF-a) levels were found to be associated with worse patient outcome in two studies (30, 46).

The relationship between CSF cytokine levels and neuropsychiatric outcome was described in four studies (37–40). These associations included: higher IL-6 and IL-8 were associated with a higher incidence of depression at 6 months (37), TNF-a levels with depression at 12 months (37), IL-5/IL-8/IL-12/TBF with

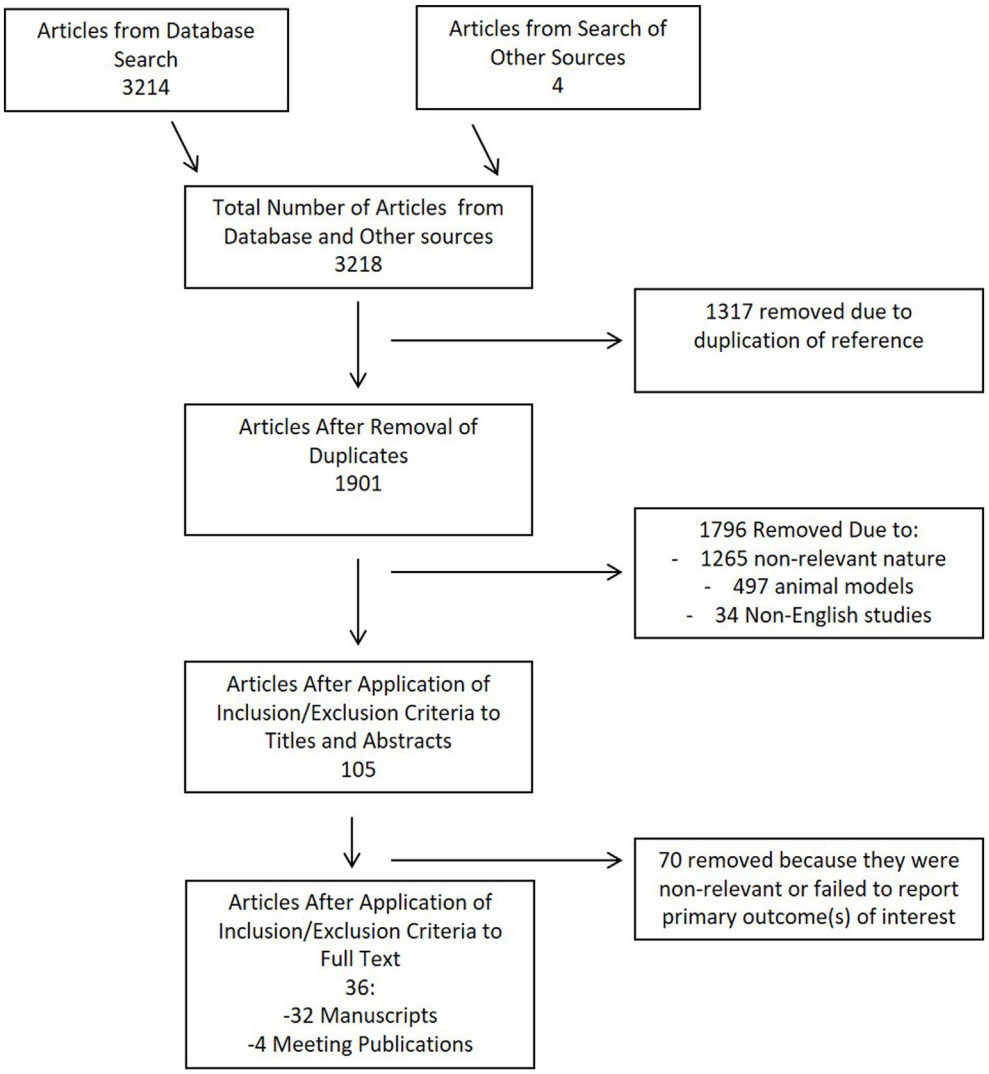

FIGURE 2 | Flow diagram of search results for cerebrospinal fluid review.

apathy at 12 months (38), TNF-a with disinhibition at 12 months (39), and sVCAM/sICAM/sFAS with depression at 6 months (40).

Neurophysiologic Outcome. Three studies discussed the correlation between CSF cytokine levels and ICP/CPP (36, 48, 52). Elevated IL-6 and IL-8 levels were associated with increased ICP and decreased CPP in one study (52). Elevated CSF IL-1b was associated with increased ICP in two studies (36, 48). One study found an association between CSF IL-6 and IL-8 levels and reduced middle cerebral artery (MCA) CBFV (51). Finally, one study found an association between IL-6 levels and the mean change in somatosensory evoked potential over 96 h recording window (49).

Nil Association Studies

Our review identified 13 studies documenting a "nil association" between CSF measured cytokines in sTBI patients and various outcomes of interest (53–65). Eleven studies reported no association between various CSF cytokines and patient outcome, as reported by in-hospital mortality or GOS at 3–6 months (53–55, 58–65). The cytokines reported within these studies varied significantly, with the most common "nil associations" reported for IL-1b, IL-6, IL-8, IL-10, TNF-a, and sTNFR. A total of 376 patients were described within these studies. Two studies reported no association between CSF cytokine measures and ICP/CPP (55, 57), while one study failed to determine an association between CSF IL-1b and post-traumatic epilepsy (56). Further detail on the "nil association" studies can be found at the bottom of **Table 4**.

Complications

Within the CMD cytokine manuscripts, the majority failed to report whether complications were considered within the data collection. Only three papers disclosed complication reporting (18, 28, 29), with two reporting "no complications" (28, 29), and

one reporting a CMD catheter malfunction in one patient (19). The complication profiles may be under-reported within the CMD studies. Complication reporting within the CSF cytokine studies was essentially non-existent, with the focus of these studies the association between CSF cytokine measures and various outcomes.

DISCUSSION

CMD Cytokines in sTBI

Our scoping systematic review completed for CMD cytokine measures in sTBI allows limited conclusions. Despite 19 publications (11–29), this literature is based on very small numbers of patients with many studies conducted on the same patient populations with banked CMD samples. However, the limited conclusions are important. First, CMD-based measurement of cytokines is feasible. Second, CMD catheter location makes a difference in the levels of cytokines measured, with peri-lesional tissue producing high levels compared to distant or healthier tissue (11, 13). Third, peaks in CMD cytokine measures may occur within the first 48–72 h for IL-1b, IL-6, and IL-8 (21–23). Interestingly, IL-10 seems to remain elevated in CMD samples through the duration of the sampling periods described (22, 26). Fourth, IL-6 levels may prove to be predictive of ongoing second insults such as ischemia (18, 19). Fifth, the data from the rhIL-1ra studies (16, 17) shows that subcutaneous rhIL-1ra leads to both an increase in CMD IL-1ra and a modulation of microglial/macrophage based cytokine profiles. Sixth, CMD IL-1b/IL-1ra/IL-6/IL-8 may be associated with poor outcome (11, 20, 29), up to 6 months post-injury. Finally, complications related to the use of CMD catheters are likely to be under-reported.

CSF Cytokines in sTBI

Our systematic review of CSF cytokines in sTBI, focused on the association between cytokine measures and patient, tissue outcome, or neurophysiology outcomes identified some interesting trends. First, a large number of heterogeneous studies correlated CSF cytokine levels with patient outcome, defined as either mortality or GOS at 6–12 months post-injury. Various large panels of cytokines were described within these studies, but the strongest associations with outcome were found for IL-1b, IL-1ra, IL-6, IL-8, IL-10, and TNF. Most studies described an association between elevated levels of these cytokines and poor GOS/increased mortality. Second, psychiatric outcome at 6–12 months post-injury appears to have some association to CSF cytokine levels (37–40). Elevated CSF IL-6, IL-8, and TNF seem to have the strongest associations with depression, apathy, and disinhibition at 6–12 months. Third, analysis of the impact of CSF cytokine levels on neurophysiologic measures is limited, with only five studies documenting such data (36, 48, 49, 51, 52). The strongest relationship identified here was the link between elevated levels of various cytokines, such as IL-6 or IL-1b, and elevated ICP (36, 48, 52). Further work is required before robust conclusions can be drawn in this area. Fourth, none of the studies explored the link between CSF cytokine measures and tissue outcome, as assessed by follow-up neuroimaging. Fifth, despite the "positive" associations found in the previously described papers, 11 manuscripts found no relationship between CSF cytokines and patient outcome (53–55, 58–65). The patient numbers in the individual studies, which reported no associations was much smaller than that in the studies describing a positive association between CSF cytokines and patient outcome (mean of 28 vs. 41 patients/study, respectively), making lack of power a possible cause of a negative result. Further patients in the "nil association" studies represented an overall smaller sample, totaling 376 patients vs. 948 patients in the "positive association" studies. Finally, complication reporting within the CSF cytokine studies was absent. Selective reporting bias here is a major concern.

Limitations

Despite the interesting results of these two systematic reviews, there are significant study limitations, which need to be highlighted. Limitations with each separate review can be found within the subsections to follow.

CMD Cytokine Review

First, there were a small number of heterogenous studies found for the CMD review, with some manuscripts reporting on the same patient populations based on banked CMD samples. Most of these studies had patient cohort with unspecified heterogeneous patterns of injury in the setting of sTBI. The exceptions were the studies describing "diffuse" TBI patients only. These drawbacks limit the generalizability of the results to all patients with sTBI. Second, the ICU and surgical therapies received by these patients during CMD sample collection/processing was quite heterogeneous and poorly reported, and could have driven substantial variation in CMD cytokine measures. Third, there were variations in CMD catheter location between studies. This could impact the CMD cytokine measures obtained and the described relationships. Fourth, complications association with CMD monitoring was seldom reported. We believe there is significant selective harms reporting. Finally, given the studies and results identified for the CMD review, there is likely a large publication bias, favoring only studies with positive results.

CSF Cytokine Review

First, there were many quite heterogeneous studies identified in the CSF cytokine review. The included papers varied by study design, number of patients, patient inclusion criteria, ICU-based therapies offered/provided to patients, blinding during outcome assessment, and primary outcome of the studies. Information regarding the relationship between CSF cytokine measures and patient outcome was often buried within the text, and often not an explicit target for the study. Furthermore, selective outcome reporting with regards to individual CSF cytokine measures and their association to patient outcome was present in many studies. Thus, the conclusions that can be drawn from these studies and the strength of associations between CSF cytokines with patient outcome/neurophysiologic outcome are limited. Second, selective outcome reporting was an issue in many studies with preference to reporting significant association(s) only, making no reference to other CSF measures and the results of statistical analysis. Third, complication reporting was concerning within

the literature identified (as mentioned above). Significant underreporting is suspected, with selective harms reporting the likely cause. Fourth, given all the above limitations and heterogeneity issues, a meta-analysis was not performed. Finally, though majority of studies report a positive association between cytokine levels and outcome, given that this is an emerging area of research, it is important to consider whether this might represent a publication bias toward positive studies.

Correlation with Clinical Parameters

Several studies attempt to correlate a specific mediator concentration with outcome. As these mediators are known to act in complex cascades and show a high degree of statistical collinearity, simple inferences cannot be made about the role of a given mediator in causing a particular outcome or relating to a clinical parameter such as ICP. As these mediators are induced by the initial traumatic insult, they are all confounded by severity of injury: it is, therefore, not surprising that a high concentration of cytokine relates to a worsened clinical parameter. Furthermore, the timing of monitoring in relation to the time of injury is not consistently reported. Several mediators, such as IL6, can have differing biological effects depending on the milieu in which they are produced (68). Finally, many mediators are known to act in concert and regulate the same downstream pathways (e.g., IL1b and IL1ra) such that measuring a mediator in isolation does not reflect its true biological role, which is time and milieu dependent.

Future Directions

Given the significant heterogeneity in both study design, patient injury patterns, ICU/surgical treatments, and CMD/CSF cytokine measures identified within both systematic reviews, there is substantial room for more investigation into this emerging area of the literature in sTBI.

Although it is tempting to simply suggest that larger studies are done to overcome the heterogeneity in injury patterns following TBI, there are significant limitations to this approach. There are an ever-expanding list of mediators available for analysis over multiple time points in a range of biological fluids and without a robust understanding of the interaction between these mediators, it is unlikely that a meaningful pattern will emerge through brute force of numbers. More refined approaches that explore within patient comparisons with multiple sites of monitoring (69), interventional studies in which specific modulation of a biological pathway (16), and more sophisticated multivariate statistical methods (14).

Some studies have attempted to relate intensive care parameters such as ICP to the cytokine and chemokine response to TBI (26). This is not a simple relationship as the time frame over which cytokines and chemokines are produced occur over several days and weeks, rather than over the minutes and hours. There is insufficient evidence to stipulate, which intensive care interventions should be applied during monitoring of inflammatory mediators; however, it is important for individual studies to report their intensive care protocols and interventions.

As CMD is necessarily focal in nature, strict reporting of the method of localization is required and ideally 2 catheter studies, 1 in peri-lesional tissue and 1 in healthy tissue provides the most informative data (quote consensus paper).

When multiple mediators are measured, multivariate statistical methods must be employed, such as multivariate projection methods in order to model the potential interactions (14, 70).

This could potentially identify cytokine patterns of coexpression in CMD and CSF, highlighting target for future studies and therapeutic targets.

One deficit in the current CMD literature is complication reporting. In part, this relates to the difficulty in apportioning complications to CMD catheter insertion specifically. As patients will have invasive monitoring for ICP monitoring and brain tissue oxygenation in any circumstance for directing clinical therapy, the additional risk of inserting CMD through an existing cranial access device is small and difficult to quantify. Nevertheless, transparency dictates that complications are reported. Standardization of the methodologies employed allows multicenter prospective evaluation of cytokines within CDM and CSF and is necessary to improve patient recruitment and aid with spreading the substantial cost of cytokine analysis among centers. Without this collaboration, the limitations with single center recruitment and costs of cytokine processing in CMD and CSF limits the ability to combine datasets across units and studies. This would allow easier compilation of data sets and may add clarity to the associations highlighted within this manuscript. Finally, a consideration of the methodological factors that determine microdialysis catheter efficiency, including choice of perfusion fluid, catheter membrane, and pump flow rate all have an impact on the result obtained.

CONCLUSION

The evaluation of CMD and CSF cytokines is an emerging area of the literature in sTBI. The two scoping systematic reviews have demonstrated a limited literature available on CMD cytokine measurement in sTBI, with some preliminary data supporting feasibility of measurement and associations between cytokines and patient outcome. Second, a number CSF cytokine levels may be associated with patient outcome at 6–12 months, including IL-1b, IL-1ra, IL-6, IL-8, IL-10, and TNF. Third, there is little to no literature to date in support of an association between CSF cytokines and neurophysiologic or tissue outcomes. Ultimately, the aim of CMD monitoring of inflammatory mediators is to reveal the underlying pathophysiology of TBI rather than as a clinical tool.

AUTHOR CONTRIBUTIONS

FZ was involved in project conception, design, systematic review searching, data extraction/tabulation, data interpretation, manuscript composition, and editing. ET was involved with data extraction/tabulation, manuscript composition, and editing. MC was involved in manuscript composition and editing. PH was involved in design, data interpretation, and manuscript editing. DM and AH was involved in design, data interpretation, manuscript writing, and editing.

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SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at http://journal.frontiersin.org/article/10.3389/fneur.2017.00331/full#supplementary-material.

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REVIEW
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Cerebrospinal Fluid and Microdialysis Cytokines in Aneurysmal Subarachnoid Hemorrhage: A Scoping Systematic Review

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Objective: To perform two scoping systematic reviews of the literature on cytokine measurement in cerebral microdialysis (CMD) and cerebrospinal fluid (CSF) in aneurysmal subarachnoid hemorrhage (SAH) patients, aiming to summarize the evidence relating cytokine levels to pathophysiology, disease progression, and outcome.

Methods: Two separate systematic reviews were conducted: one for CMD cytokines and the second for CSF cytokines.

Data sources: Articles from MEDLINE, BIOSIS, EMBASE, Global Health, Scopus, Cochrane Library (inception to October 2016), reference lists of relevant articles, and gray literature were searched.

Study selection: Two reviewers independently identified all manuscripts utilizing predefined inclusion/exclusion criteria. A two-tier filter of references was conducted.

Data extraction: Patient demographic and study data were extracted to tables.

Results: There were 9 studies identified describing the analysis of cytokines *via* CMD in 246 aneurysmal SAH patients. Similarly, 20 studies were identified describing the analysis of CSF cytokines in 630 patients. The two scoping systematic reviews demonstrated the following: (1) limited literature available on CMD cytokine measurement in aneurysmal SAH with some preliminary data supporting feasibility of measurement and potential association between interleukin (IL)-6 and patient outcome. (2) Various CSF measured cytokines may be associated with patient outcome at 3–6 months, including IL-1ra, IL-6, IL-8, and tumor necrosis factor-alpha. (3) There is a small literature body supporting an association between acute/subacute CSF transforming growth factor levels and the development of chronic hydrocephalus at 2–3 months.

Conclusion: The evaluation of CMD and CSF cytokines is an emerging area of the literature in aneurysmal SAH. Further large prospective multicenter studies on cytokines in CMD and CSF need to be conducted.

Keywords: subarachnoid hemorrhage, systematic review, cytokines, cerebrospinal fluid, micordialysis

INTRODUCTION

Inflammation in the setting of aneurysmal subarachnoid hemorrhage (SAH) is believed to be a potential driver of many secondary insults in this often critically ill population (1–4). As with animal models of stroke, inflammatory mediators have been associated with loss of salvageable ischemic penumbra, infarct propagation, and cerebral edema (5–8). It has been proposed that various inflammatory cytokines are associated with secondary injury and pathological processes post-aneurysmal SAH (1, 2).

Within the aneurysmal SAH population, numerous studies have been published associating serum inflammatory markers with patient outcome and the risk of cerebral vasospasm/delayed ischemic neurological deficits (DINDs) (1, 3, 4). Furthermore, analysis of cerebrospinal fluid (CSF) cytokines has demonstrated an association between interleukin (IL)-6 and tumor necrosis factor-alpha (TNF-a) with the risk of cerebral vasospasm and DIND through an extensive systematic review/meta-analysis of the available literature (9). Of note, this systematic review focused only on the relationship IL-6 and TNF-a with vasospasm, without any comment or consideration of other important primary outcomes such as patient morbidity/mortality.

Aside from the above-mentioned associations, the remaining literature on inflammatory cytokines in aneurysmal SAH is scattered and scarce. In particular, the literature on cytokines in cerebral microdialysis (CMD) samples from aneurysmal SAH patients is very limited (10–18). In addition, apart from the systematic review on the association between CSF IL-6 and TNF-a with vasospasm/DIND (9), there is limited literature on the association between CSF cytokines and other relevant endpoints (10, 19–37) such as patient functional outcome, neurophysiologic outcome, chronic hydrocephalus/ventriculoperitoneal shunt (VPS) dependency, and tissue fate.

The goal of this project was to produce a scoping systematic review of the literature on both CMD and CSF cytokines in aneurysmal SAH. There were two main focuses for this article: (1) to provide a comprehensive scoping systematic review of the literature on CMD cytokines in aneurysmal SAH; (2) produce a scoping systematic review evaluating the association between CSF cytokines and the following outcomes (excluding vasospasm/DIND): patient functional outcome, neurophysiologic outcome, chronic hydrocephalus/VSP dependency, and tissue outcome.

METHODS

Two separate scoping systematic reviews were conducted, using the methodology outlined in the Cochrane Handbook for Systematic Reviewers (38). Data were reported following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (39). The review questions and search strategy were decided upon by the primary author (Frederick A. Zeiler) and supervisors (Adel Helmy and David K. Menon).

This article was conducted in concert with a similar review on cytokines in CMD and CSF for severe traumatic brain injury (TBI) patients, which is currently unpublished and under review (40).

Search Question and Population of Interest

Given that two separate systematic reviews were conducted, one for CMD cytokines and the other for CSF-based cytokines, two distinct questions were posed. The lack of literature identified through a preliminary search of PubMed led us to conduct a scoping review for the CMD cytokine search, with the attempt to identify all studies in this area to date. The larger literature base for CSF cytokines in aneurysmal SAH led us to narrow our question for this scoping review, focusing on relevant outcomes (see below). The questions posed for this scoping systematic review were as follows:

1. What literature has been published on CMD of cytokines in aneurysmal SAH?
2. Is there literature to suggest an association between CSF-based cytokine measures in aneurysmal SAH and patient outcome, chronic hydrocephalus/shunt dependency, neurophysiologic outcome, or tissue outcome?

For the CMD cytokine scoping review, all articles describing microdialysis-based cytokine measures in humans with aneurysmal SAH were included to provide a comprehensive overview.

For the CSF cytokine review, the primary outcome measures of interest were documented association between CSF measured cytokines and patient outcome, chronic hydrocephalus/shunt dependency, neurophysiologic outcome (as measured *via* intensive care unit (ICU)-based monitoring, intracranial pressure/cerebral perfusion pressure, brain tissue oxygen monitoring ($PbtO_2$), thermal diffusion assessment of cerebral blood flow (CBF), transcranial Doppler (TCD) measure of cerebral blood flow velocity, any neuroimaging-based assessment of CBF/perfusion, and electrophysiology), and tissue outcome [as assessed on follow-up neuroimaging by either computed tomography (CT) or magnetic resonance imaging]. Any outcome score or mention of morbidity/mortality within the studies was deemed acceptable for documentation of patient outcome. Secondary outcome measures were complications associated with CSF monitoring of cytokines. Of note, cerebral vasospasm and DIND were specifically excluded as a primary outcome for the CSF cytokine review given a recently conducted systematic review published on this exact relationship (9).

Acceptable cytokines in CMD or CSF included IL-1a, IL-1b, IL-1ra, IL-2, sIL-2ra, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-11, IL-12, IL-12p70, IL-13, IL-14, IL-15, IL-16, IL-17, inducible protein-10, eotaxin, TNF, interferon gamma, monocyte chemoattractant proteins (MCP), macrophage inflammatory proteins (MIPs), transforming growth factor (TGF), nerve growth factor, brain-derived neurotrophic factor, glial-derived neurotrophic factor, soluble tumor necrosis factor receptor (sTNFR), granulocyte macrophage colony-stimulating factor (GM-CSF), sFAS, soluble vascular cell adhesion molecule-1, soluble intracellular adhesion molecule-1, platelet-derived growth factor, RANTES, macrophage-derived chemokine (MDC), Flt3, fractalkine, and fibroblast growth factor receptor.

Inclusion/Exclusion Criteria

CMD Cytokine Review

Inclusion criteria: all studies including human subjects with aneurysmal SAH, any study size, any age category, CMD analysis for cytokines, and mention of any outcome (patient based or otherwise; excluding vasospasm/DIND). Exclusion criteria: non-English studies, only non-cytokine/chemokine inflammasome proteins measured, animal studies, non-aneurysmal SAH, and unconfirmed/angio-negative SAH.

CSF Cytokine Review

Inclusion criteria were as follows: all studies including human subjects with aneurysmal SAH, studies with 10 or more patients, any age category, CSF analysis for cytokines, and documentation either: patient functional outcome, neurophysiologic outcome, or tissue outcome in relation to CSF cytokine measures. Exclusion criteria were as follows: non-English studies, only non-cytokine/chemokine inflammasome proteins measured, animal studies, non-aneurysmal SAH, unconfirmed/angio-negative SAH, and studies of less than 10 patients. Also excluded were studies focused only on the outcome of cerebral vasospasm/DIND (as this has been explored in a recent systematic review and meta-analysis).

Search Strategies

MEDLINE, BIOSIS, EMBASE, Global Health, SCOPUS, and Cochrane Library from inception to October 2016 were searched using individualized search strategies. The search strategy for the CMD scoping systematic review using MEDLINE can be seen in Appendix A in Supplementary Material, with a similar search strategy utilized for the other databases. Further, the search strategy for the CSF scoping systematic review using MEDLINE can be seen in Appendix B in Supplementary Material, with similar strategies employed for the other databases.

In addition, we surveyed relevant meeting proceedings for the last 5 years looking for ongoing and unpublished work based on the cytokine analysis *via* CMD or CSF in aneurysmal SAH patients. We elected to include published meeting proceedings to provide as comprehensive of a scoping systematic review as possible. It is acknowledged that the quality of evidence derived from such pseudopeer-reviewed meeting publications is limited. However, given that cytokine research in SAH is relatively "new" and our goal was to produce a systematically conducted scoping review on the topic, we elected to include them to be comprehensive. The meeting proceedings of the following professional societies were searched: Canadian Neurological Sciences Federation, American Association of Neurological Surgeons, Congress of Neurological Surgeons, European Neurosurgical Society, World Federation of Neurological Surgeons (WFNS), American Neurology Association, American Academy of Neurology, European Federation of Neurological Science, World Congress of Neurology, Society of Critical Care Medicine, Neurocritical Care Society, European Society for Intensive Care Medicine, World Federation of Societies of Intensive and Critical Care Medicine, American Society for Anesthesiologists, World Federation of Societies of Anesthesiologist, Australian Society of Anesthesiologists, International Anesthesia Research Society, Society of Neurosurgical Anesthesiology and Critical Care, Society for Neuroscience in Anesthesiology and Critical Care, Japanese Society of Neuroanesthesia and Critical Care, and the College of Intensive Care Medicine Annual Scientific Meeting (Australia), World Stroke Organization, UK Stroke Forum, International Stroke Conference, European Stroke Society, Canadian Stroke Congress, SMART Stroke Group, and the Australian Stroke Society.

Finally, reference lists of any review articles on CSF or CMD cytokines were reviewed for any missed relevant studies.

Study Selection

Utilizing two reviewers, a two-step review of all articles returned by our search strategies was performed. First, the reviewers independently (Frederick A. Zeiler and Eric Peter Thelin) screened titles and abstracts of the returned articles to decide if they met the inclusion criteria. Second, full text of the chosen articles was then assessed to confirm if it met the inclusion criteria and that the primary outcome of interest was reported in the studies (Frederick A. Zeiler and Eric Peter Thelin). Any discrepancies between the two reviewers were resolved by a third reviewer if needed (Adel Helmy or David K. Menon).

Data Collection

Data were extracted from the selected articles and stored in an electronic database. Data fields included type of study, article location, number of patients, patient demographics, aneurysm characteristics/treatment, Hunt and Hess (H + H) clinical grade (41), World Federation of Neurological Surgeons (WFNS) clinical grade (42), Fisher CT grade (43), ICU therapies applied, CMD/CSF substrate measured, CMD/CSF measurement details (probe tissue location and sampling frequency), outcome measure described (patient, neurophysiologic, and tissue), association between CMD/CSF cytokine measure to outcome, and complications. Complications of interest for the CSF studies were any related to ventriculostomy: misplacement, tract hemorrhage, infection, and extra-axial hemorrhage/collection formation. All data for both the CSF and CMD cytokine studies can be found in **Tables 1–4**.

Bias Assessment

As the goal of this study was to produce a systematically conducted scoping review of the available literature on CMD and CSF cytokine measures in aneurysmal SAH, formal bias assessment was not done. Our desire was to produce a comprehensive overview of the current literature on the topic of CMD/CSF cytokines in aneurysmal SAH. Formal evidence grading was not conducted (given the limited and heterogenous literature body), and thus, we deemed formal bias risk assessment unnecessary for this emerging area of literature, which clearly suffers from standard biases associated with new areas of clinical research.

Statistical Analysis

A meta-analysis was not performed in this study due to the heterogeneity of data and study design within the articles identified.

TABLE 1 | CMD cytokine study characteristics and patient demographics.

Reference	Number of patients	Study type	Article location	Mean age (years)	Patient characteristics	Primary and secondary goal of study
Graetz et al. (10)	24	Prospective observational	Manuscript	50 years (range: 43.5–62 years)	aSAH Admission WFNS: I–III in 14 IV–V in 10 Mean Fisher CT Score: 4 (range: 3–4) Aneurysm location ICA/MCA = 5/13 ACA/PComm = 3/3	*Primary*: to evaluate the pattern of IL-6 expression in CMD, CSF, and serum *Secondary*: compare IL-6 expression to ICP, CMD-defined ischemia (LPR > 30, glycerol > 80 μmol/L), and outcome
Hanafy et al. (11)*	14	Prospective observational	Manuscript	48 years (range: 34–59 years)	aSAH Admission WFNS: IV in 1 V in 13 Aneurysm locations: ICA (4); MCA (3); ACA (6); VA (1)	*Primary*: to measure CMD TNF-a post-aSAH *Secondary*: to determine if clinical characteristics predict CMD TNF-a levels
Hanafy et al. (12)*	10	Retrospective case series	Manuscript	45.5 years (range: 27–65 years)	aSAH Admission H + H: 2 in 1 3 in 1 4 in 3 5 in 5 Fisher CT: Median = 3 (range: 2–4) Aneurysm locations: AComm (3); ICA (3); MCA (2); PCA (1); VA (1)	*Primary*: to determine the correlation between CMD TNF-a and radiographic vasospasm as per CTA/DSA *Secondary*: none mentioned
Helbok et al. (13)	26	Prospective observational	Manuscript	55 years (range: 47–67 years)	aSAH Admission H + H: 2 in 2 3 in 6 4 in 2 5 in 16 Aneurysm location: unclear locations	*Primary*: to measure CMD IL-6 and CMD MMP-9 and determine the relationship to outcome *Secondary*: to determine the temporal course of IL-6 post-aSAH
Mellergård et al. (14)	21 with aSAH (38 total in study with mixed pathology)	Prospective observational	Manuscript	Unknown	aSAH No specifics on clinical status or aneurysms	*Primary*: to evaluate CMD cytokine profiles immediately after insertion of the CMD catheter *Secondary*: none mentioned
Mellergård et al. (15)*	88 with aSAH (Total 145 patients with mixed pathology)	Retrospective case series	Manuscript	Unknown	aSAH No specifics on clinical status or aneurysms	*Primary*: to determine the CMD cytokine responds to aSAH *Secondary*: none mentioned
Mellergård et al. (16)*	88 with aSAH (total 145 patients with mixed pathology)	Retrospective case series	Manuscript	Unknown	aSAH No specifics on clinical status or aneurysms	*Primary*: to determine the CMD cytokine response to aSAH *Secondary*: none mentioned

(*Continued*)

TABLE 1 | Continued

Reference	Number of patients	Study type	Article location	Mean age (years)	Patient characteristics	Primary and secondary goal of study
Sarrafzadeh et al. (17)	38	Prospective observational	Manuscript	53.1 years (range: unknown)	aSAH—29% with acute focal deficits on admission Admission WFNS scores: I in 12 II in 7 III in 3 IV in 7 V in 9 Mean Fisher CT score = 4 Aneurysm locations: no specific given	*Primary*: to measure CMD, CSF and serum IL-6 post-aSAH *Secondary*: correlate to clinical course
Schiefecker et al. (18)	25	Prospective observational	Manuscript	Unknown	aSAH—poor grade	*Primary*: to evaluate CMD IL-6 levels post-aSAH and determine the association DIND and outcome at 3 months *Secondary*: determine probe relationship to IL-6 expression

**Studies from the same Authors and Center – there may be duplicated patient information.*
aSAH, aneurysmal subarachnoid hemorrhage; H + H, Hunt and Hess; WFNS, World Federation of Neurological Surgeons; CT, computed tomography; AComm, anterior communicating artery; PComm, posterior communicating artery; MCA, middle cerebral artery; ACA, anterior cerebral artery; ICA, internal cerebral artery; VA, vertebral artery; VBA, vertebrobasilar; PICA, posterior inferior cerebellar artery; CMD, cerebral microdialysis; CSF, cerebrospinal fluid; ICP, intracranial pressure; LPR, lactate:pyruvate ratio; IL, interleukin; MABP, mean arterial blood pressure; DC, decompressive craniectomy; DIND, delayed ischemic neurological deficit; PCA, principle component analysis.

TABLE 2 | CSF cytokine study characteristics and patient demographics.

Reference	Number of patients	Study type	Article location	Mean age (years)	Patient characteristics	Primary and secondary goal of study
Chou et al. (19)	29	Prospective observational	Meeting abstract	Unknown	aSAH Unknown admission clinical/radiologic grades No data on aneurysm characteristics	*Primary*: to determine the association between CSF cytokines with vasospasm and patient outcome *Secondary*: none mentioned
Graetz et al. (10)	24	Prospective observational	Manuscript	50 years (range: 43.5–62 years)	aSAH Admission WFNS: I–III in 14 IV–V in 10 Mean Fisher Score: 4 (range: 3–4) Aneurysm Location: ICA/MCA = 5/13 ACA/PComm = 3/3	*Primary*: to evaluate the pattern of IL-6 expression in CMD, CSF, and serum *Secondary*: compare IL-6 expression to ICP, CMD-defined ischemia (LPR > 30, glycerol > 80 μmol/L), and outcome
Gruber et al. (20)	44	Prospective observational	Manuscript	51.3 years (range: 24–80 years)	aSAH Admission H + H: I = 2 II = 4 III = 15 IV = 19 V = 4 Aneurysm location: Ant Circ = 30 Post Circ = 14	*Primary*: to measure CSF and serum cytokines. Determine any association to outcome. *Secondary*: none mentioned

(*Continued*)

TABLE 2 | Continued

Reference	Number of patients	Study type	Article location	Mean age (years)	Patient characteristics	Primary and secondary goal of study
Höllig et al. (21)	46 (total 81; only 46 with CSF sampling)	Prospective observational	Manuscript	53.8 years (range: 29–87 years)	aSAH Admission WFNS: Mean = 2.96 Admission Fisher Score: Mean = 3.31 Aneurysm location: AComm = 26 MCA = 17 ICA = 19 BA = 7 "other" = 12	*Primary*: to determine the relation of serum and CSF cytokines with discharge outcome. *Secondary*: to determine the relation of serum and CSF cytokines with 6-month outcome
Mathiesen et al. (22)	22	Prospective observational	Manuscript	51.3 years (range: 32–77 years)	aSAH Admission H + H: I = 0 II = 14 III = 3 IV = 5 Fisher CT score: Not specified Aneurysm location: AComm = 9 MCA = 6 PComm = 3 ICA = 2 VA/VAB = 2	*Primary*: to measure CSF cytokines in SAH patients and correlate to outcome and vasospasm. *Secondary*: none mentioned
Nakahara et al. (23)	39	Prospective observational	Manuscript	62.9 years (range: 52–71)	aSAH Admission H + H: Range = 2–4 Fisher CT score: Range = 3–4 Aneurysm location: AComm = 12 MCA = 12 ICA/PComm = 11 ACA = 2 BA = 2	*Primary*: to determine the correlation between HMGB-1 and other cytokines with patient outcome at 3 months *Secondary:* none mentioned
Niwa et al. (24)	10	Prospective observational	Manuscript	57 years (range: 41–75 years)	aSAH Admission H + H: I = 0 II = 6 III = 2 IV = 2 V = 0 Fisher CT score: I = 0 II = 0 III = 7 IV = 3 Aneurysm location: AComm = 6 MCA = 2 ICA/PComm = 2	*Primary:* to measure various CSF cytokines post-SAH. Determine association to patient outcome *Secondary:* none mentioned

(*Continued*)

TABLE 2 | Continued

Reference	Number of patients	Study type	Article location	Mean age (years)	Patient characteristics	Primary and secondary goal of study
Provencio et al. (25)	14	Prospective observational	Meeting abstract	Unknown	aSAH Unknown admission clinical/radiologic grades No data on aneurysm characteristics	*Primary:* to determine the relationship between serum and CSF cytokines with patient outcome *Secondary:* none mentioned
Sokół et al. (26)	10	Prospective observational	Manuscript	61.1 years (range: unknown)	aSAH Admission H + H: Mean = 4 (range: 4–4) Admission Fisher CT score: Mean = 4 (range: 2–4) Aneurysm location: AComm = 4 ACA = 2 BA = 2 PCA = 1 PICA = 1	*Primary:* to determine the association between CSF HMGB-1 and patient outcome *Secondary:* none mentioned
Wada et al. (27)	45	Prospective observational	Meeting abstract	Unknown	aSAH Unknown admission clinical/radiologic grades No data on aneurysm characteristics	*Primary:* to determine the association between serum/CSF G-CSF and both vasospasm and patient outcome *Secondary:* none mentioned
SHUNT DEPENDENCY STUDIES						
Douglas et al. (28)	20	Prospective observational	Manuscript	47 years (range: 23–64 years)	aSAH Admission WFNS: I = 5 II = 4 III = 1 IV = 2 V = 6 ND = 2 Admission Fisher CT score: I = 1 II = 2 III = 3 IV = 11 ND = 3 Aneurysm location: ACA = 3 ICA = 2 PCA = 1 PICA = 3 BA = 1 Traumatic = 1 ND = 8	*Primary:* to determine the association between CSF TGF-b1 and TGF-b2 with shunt dependency post-aSAH *Secondary:* to compare CSF cytokines in aSAH and non-hemorrhage hydrocephalus patients
Kitazawa and Tada (29)	24	Prospective observational	Manuscript	61.2 years (range: 39–78 years)	aSAH unknown admission clinical grades Admission Fisher CT grade: II = 5 III = 14 IV = 3 Aneurysm locations: unknown	*Primary:* to determine the association between CSF TGF-b1 levels and the development of CT-defined hydrocephalus and VPS dependency *Secondary:* none mentioned

(*Continued*)

TABLE 2 | Continued

Reference	Number of patients	Study type	Article location	Mean age (years)	Patient characteristics	Primary and secondary goal of study
Takizawa et al. (30)	36	Prospective observational	Manuscript	60.3 years (range: 39–81 years)	aSAH Unknown admission clinical/radiologic grades No data on aneurysm characteristics	*Primary:* to determine the association between CSF measured cytokines and the development of hydrocephalus *Secondary:* compare levels to non-hemorrhagic controls (n = 11)
Wostrack et al. (31)	69	Prospective observational	Manuscript	57 years (range: 21–80 years)	aSAH Admission H + H: I = 3 II = 17 III = 25 IV = 12 V = 12 Unclear Fisher grades Aneurysm locations: Anterior = 23 Middle = 16 ICA = 16 Posterior = 10	*Primary:* to determine the association between CSF cytokines and shunt dependency *Secondary:* none mentioned
NIL ASSOCIATION STUDIES						
Gaetani et al. (32)	31	Prospective observational	Manuscript	52.6 years (range: unknown)	aSAH Admission WFNS: 1–2 = 24 3–4 = 7 Unclear Fisher grade and aneurysm Locations	*Primary:* to determine the association between various CSF cytokines and the development of vasospasm
Kaestner and Dimitriou (33)	27 (42 total; but non-aneurysmal IVH in 15 patients)	Prospective observational	Manuscript	52.2 years (range: unknown)	aSAH Unknown admission clinical/radiologic grades Unknown aneurysm locations	*Primary*: to determine the association between CSF TGF-b1 and hydrocephalus *Secondary*: to compare CSF TGF-b1 with non-hemorrhagic communicating hydrocephalus (n = 7)
Kim et al. (34)	51 (77 total; only 51 with CSF samples)	Prospective observational	Manuscript	Unclear mean and range	aSAH Unknown admission clinical/radiologic grades No data on aneurysm characteristics	*Primary*: to determine the association between serum and CSF MIP-1 with patient outcome *Secondary*: to determine the association between serum and CSF MIP-1 with vasospasm
Kwon and Jeon (35)	12 (19 patients total; 12 with CSF)	Prospective observational	Manuscript	46.5 years (range: 29–65 years)	aSAH Admission H + H: I = 2 II = 6 III = 8 IV = 3 Admission Fisher score: I = 3 II = 5 III = 9 IV = 2 Aneurysm characteristics: ACom = 8 MCA = 3 PCom = 5 ICA = 1 ACA = 1 VA = 1	*Primary*: to determine the relationship between serum/CSF cytokines and vasospasm. Also determine the link to patient outcome *Secondary*: none mentioned

(*Continued*)

TABLE 2 | Continued

Reference	Number of patients	Study type	Article location	Mean age (years)	Patient characteristics	Primary and secondary goal of study
Shoch et al. (36)	64	Prospective observational	Manuscript	55 years (range: 29–77 years)	aSAH Admission WFNS: I = 9 II = 12 III = 4 IV = 25 V = 14 Admission Fisher CT Score: I = 0 II = 4 III = 32 IV = 28 Aneurysm Location: ACA = 23 MCA = 12 AComm = 15 VBA = 14	*Primary*: to determine the association between CSF IL-6 and vasospasm *Secondary*: none mentioned
Singh et al. (37)	13	Prospective RCT	Manuscript	54 years (range: 40–69 years)	aSAH Admission WFNS: I = 1 II = 5 III = 0 IV = 3 V = 4 Admission Fisher CT score: III = 3 IV = 10 Aneurysm location: Anterior = 9 Posterior = 4	*Primary*: to evaluate the use of IV IL-1ra in aSAH and evaluated CSF cytokine response *Secondary*: to evaluate patient outcome

aSAH, aneurysmal subarachnoid hemorrhage; H + H, Hunt and Hess; WFNS, World Federation of Neurological Surgeons; RCT, randomized control trial; CT, computed tomography; VPS, ventriculoperitoneal shunt; IVH, intraventricular hemorrhage; AComm, anterior communicating artery; PComm, posterior communicating artery; MCA, middle cerebral artery; ACA, anterior cerebral artery; ICA, internal cerebral artery; VA, vertebral artery; VBA, vertebrobasilar; PICA, posterior inferior cerebellar artery; CMD, cerebral microdialysis; CSF, cerebrospinal fluid; ICP, intracranial pressure; LPR, lactate:pyruvate ratio; IL, interleukin; TGF, transforming growth factor; G-CSF, granulocyte colony-stimulating factor; HMGB, high-mobility group box; MABP, mean arterial blood pressure; DC, decompressive craniectomy; PCA, principle component analysis.

RESULTS

Search Strategy Results

CMD Cytokine Search

Search strategy results for CMD cytokines in aneurysmal SAH can be seen within the flow diagram in **Figure 1**. At total of 60 references were returned, all coming from the database search and none identified *via* meeting proceeding searches. After duplicate removal, there were 30 articles left for the assessment *via* the first filtering of title and abstract content. Thirteen articles passed the first filter, requiring acquisition of the full manuscript to assess inclusion eligibility. Through assessment of the full articles, nine manuscripts were deemed eligible for inclusion in the final CMD systematic review. No articles were added from the reference sections of either review papers or the parent manuscripts included in the systematic review.

CSF Cytokine Search

The search strategy flow diagram for the CSF cytokine scoping systematic review can be seen in **Figure 2**. Overall, 516 articles were identified, with 513 from the database search and 3 from published meeting proceedings. Two hundred and eighty duplicates removed, leaving 236 references to review in the first filter. After implementation of the first filter, 61 articles were selected for assessment of their full manuscripts. One additional reference was added from the reference sections of review papers. After the second filter of full manuscripts, 20 articles were deemed eligible for final inclusion in the CSF systematic review. Remaining articles were excluded due to non-relevance.

Patient/Study Demographics

CDM Cytokine Review

All of the nine articles included in the CMD cytokine portion of the systematic review all were formal manuscript publications

TABLE 3 | CMD cytokine measures and outcomes.

Reference	Catheter location and measured CMD cytokines	Interventional therapies applied during measurement	Primary outcome	Secondary outcome	Complications to CMD	Conclusions
Graetz et al. (10)	Inserted into territory of aneurysm (whether healthy or injured) *IL-6* Unclear pooled analysis over a 10 days period Ringer's perfusate utilized	Protocolized therapy for monitoring and Tx of ICP; 3 patients underwent DC 20 patients clipped	IL-6 in CSF and CMD were typically higher than in serum	*ICP*: CMD and CSF IL-6 levels were higher in the high ICP patients, with significant for CMD samples ($p = 0.029$) CMD IL-6 levels increased after day 4 in the high ICP group *Ischemia (as per LPR > 30 and glycerol > 80 μmol/L):* no correlation between CMD, CSF, or serum IL-6 and ischemia *Outcome (dichotomized GOS at 3 and 6 months):* high CMD IL-6 levels were associated with poor outcome ($p = 0.06$)	Not specified	1. IL-6 levels in CSF, CMD, and serum are elevated after aSAH 2. CMD IL-6 levels are higher in those with ICP issues 3. No correlation between CMD IL-6 and ischemia 4. Potential weak association between CMD IL-6 levels and outcome at 3 and 6 months
Hanafy et al. (11)*	Unclear tissue location *TNF-a* q6 hour sampling for unclear duration Isotonic crystalloid perfusate	Unclear DIND monitoring; various ICP/CPP directed therapies Unclear Surgical Tx	TNF-a as measured *via* CMD is feasible and elevated post-SAH	Only the existence of IVH and aneurysm size >6 mm was correlated to TNF-a levels in CMD	Not specified	TNF-a is elevated in CMD post-aSAH IVH and large aneurysm size is associated with elevated CMD TNF-a levels
Hanafy et al. (12)*	Unclear tissue location *TNF-a* q6 hour sampling for unclear duration Isotonic crystalloid perfusate	Not specified; unclear surgical Tx	Increase CMD TNF-a between days 4 and 6 post-hemorrhage was associated with a worse radiographic vasospasm index ($p < 0.01$) No comments were made on the relationship to DIND secondary to cerebral vasospasm	N/A	Not specified	Elevated CMD TNF-a levels may correlation with radiographic vasospasm
Helbok et al. (13)	"Right frontal" in mixed tissue states *IL-6* Unclear sampling interval Isotonic crystalloid perfusate	Protocolized investigations for vasospasm, otherwise unclear ICU treatments 18 patients clipped; some had DC	*3-month mRS* CMD IL-6 and LPR were higher in those patients with worse mRS at 3 months ($p = 0.01$)	No correlation between IL-6 and MMP-9 *via* CMD IL-6 was highest initially after bleed and in cases where rebleed occurred	Not specified	CMD IL-6 may be associated with outcome at 3 months
Mellergård et al. (14)	Mixed locations; some patients with 2 catheters (unclear which patients) *IL-1b, IL-6, IL-8, FGF-2, MIP-1β, RANTES, VEGF, IL-10* q6 hour pooled samples for 36 h Ringer-Dextran 60 perfusate	Not specified	IL-1b peaked in the first 12 h period IL-6 peaked after 12 h post-insertion IL-8 peaked within the first 6 h post-insertion MIP-1b peaked within the first 6 h post-insertion	N/A	Not specified	CMD catheter insertion leads to IL-1b/IL-6/IL-8/MIP-1b within the first 6–12 h, which then decrease during the subsequent time afterward

(*Continued*)

TABLE 3 | Continued

Reference	Catheter location and measured CMD cytokines	Interventional therapies applied during measurement	Primary outcome	Secondary outcome	Complications to CMD	Conclusions
			FGF-2 peaked within the first 6 h post-insertion IL-10, VEGF, and RANTES did not show a temporal profile			
Mellergård et al. (15)*	Some with paired catheters (1 perilesonal; 1 healthy tissue)—used the catheter with highest glycerol levels for measuring cytokines	Not Specified	IL-1b increased during the first 48 h and then decreased	N/A	Not specified	IL-1b and IL-6 display a peak elevation during the first 48 h post-aSAH
	IL-1b, IL-6, IL-10		IL-6 increased over the first 48 h and then decreased			
	q6 hour pooled analysis for 7 days	65 patients clipped	IL-10 remained elevated throughout the measurement period			IL-10 remains elevated through the first 7 days post-aSAH
	Ringer-Dextran 60 perfusate		No comments on cytokine profiles in clipping vs. coiling patients were made			
Mellergård et al. (16)*	Paired catheters (1 perilesonal; 1 healthy tissue)—used the catheter with highest glycerol levels for measuring cytokines	Not specified	FGF-2 levels peaked at day 3 post-TBI	N/A	Not specified	FGF-2/VEGF levels peaked on days 3 and 2 post-aSAH
	FGF-2, VEGF q6 hour pooled analysis for 7 days Ringer-Dextran 60 perfusate	65 patients clipped	VEGF levels peaked on day 2 post-aSAH and were higher in those whom underwent surgical clipping			Surgical clipping changes the inflammatory mediator expression in CMD
Sarrafzadeh et al. (17)	Single catheter in territory where aneurysm located	Not specified	IL-6 levels in CMD and CSF were higher than serum	N/A	Not specified	IL-6 levels are elevated in CMD and CSF post-aSAH
	IL-6	Unclear surgical Tx for aneurysm	IL-6 levels in CSF, CMD, and serum were higher in those with symptomatic vasospasm but was not predictive			
	2–3 times daily for 10 days	Some received DC	However, CMD and CSF IL-6 levels were higher in those presenting with acute deficits and predicted the development of further DIND secondary to vasospasm on day 7 post-bleed ($p = 0.025$)			IL-6 CMD levels may be predictive of DIND secondary to vasospasm in those presenting with acute deficits
	Ringer's perfusate	10 developed DIND secondary to vasospasm—Tx unclear				

(*Continued*)

TABLE 3 | Continued

Reference	Catheter location and measured CMD cytokines	Interventional therapies applied during measurement	Primary outcome	Secondary outcome	Complications to CMD	Conclusions
Schiefecker et al. (18)	Mixed locations	Not specified	Patients were categorized into low-grade or high-grade inflammation based on median CMD IL-6 levels	*CMD probe location*: peri-lesional location associated with high IL-6 levels ($p = 0.002$)	Not specified	CMD IL-6 levels are higher in peri-lesional areas and in patients with ICH post-aSAH
	IL-6		Brain extracellular TAU-protein levels ($p = 0.001$), metabolic distress, and delayed cerebral infarction ($p = 0.001$) were linked to high-grade neuroinflammation			
	Unclear sampling interval Unknown perfusate		*Outcome: high*-grade neuroinflammation was a predictor for worse outcome three months after ictus, independently from probe location, initial H + H grade and age ($p = 0.01$)	*ICH:* presence of ICH was associated with elevated IL-6 levels ($p = 0.003$)		CMD IL-6 levels may be associated with DIND and outcome at 3 months

**Studies from the same Authors and Center – there may be duplicated patient information.*
aSAH, aneurysmal subarachnoid hemorrhage; ICH, intracerebral hemorrhage; IVH, intraventricular hemorrhage; mRS, modified Rankin scale; GOS, Glasgow outcome scale; H + H, Hunt and Hess; CMD, cerebral microdialysis; LP, lumbar puncture; ICP, intracranial pressure; CSF, cerebrospinal fluid; LPR, lactate:pyruvate ratio; DC, decompressive craniectomy; IL, interleukin; a, alpha; b, beta; g, gamma; TNF, tumor necrosis factor; INF, interferon; MIP, macrophage inflammatory proteins; TNFR, tumor necrosis factor receptor; VEGF, vascular endothelial growth factor; FGF, fibroblast growth factor; DIND, delayed ischemic neurological deficit; TBI, traumatic brain injury; ICU, intensive care unit.

TABLE 4 | CSF cytokine measures and outcomes.

Reference	Interval of cytokine measure	CSF cytokines measured	Interventional therapies applied during measurement	Outcome of interest	Other interesting CSF cytokine-related outcomes	Conclusions
PATIENT FUNCTIONAL OUTCOME						
Chou et al. (19)	EVD-based sampling Unclear sampling interval	*IL-2, IL-4, IL-5, IL-6, EGF, fractalkine, PDGF-AA*	Not specified Unclear clip vs. coil numbers	*Outcome assessed via mRS at 6 months:* IL-4 ($0 = 0.02$) associated with good 6-month mRS No association between CSF cytokines and vasospasm	N/A	CSF IL-4 may be associated with 6 month outcome
Graetz et al. (10)	EVD-based sampling	*IL-6*	Protocolized therapies directed toward ICP/CPP and vasospasm monitoring *via* TCD	*Outcome assessed via dichotomized GOS at 3 and 6 Months (good = 4 or 5; poor = 1–3)*	*Vasospasm:*	CSF IL-6 may be associated with outcome at 6 months
	Q8 hours for days 0–4 Q12 hours for days 5–10		Unclear clip vs. coil numbers	CSF IL-6 on days 5–9 post-bleed were associated with 6 month outcome ($p < 0.05$)	No correlation between CSF IL-6 and DIND *Micodialysis based Ischemia (LPR > 30, glutamate > 80 μmol/L):* No association between CSF IL-6 and CMD-based ischemia	

(*Continued*)

TABLE 4 | Continued

Reference	Interval of cytokine measure	CSF cytokines measured	Interventional therapies applied during measurement	Outcome of interest	Other interesting CSF cytokine-related outcomes	Conclusions
Gruber et al. (20)	EVD-based sampling Day 1, 3–5, 6–8, 9–11 post-bleed	*sTNFR-I, IL-1ra, TNF-a, TNF-b, IL-1a, IL-1b*	15 patients clipped Otherwise not specified	*Outcome assessed via GOS at 6 months:* Elevated CSF IL-1ra ($p < 0.001$), sTNFR ($p = 0.02$), and IL-6 ($p = 0.001$) were associated with outcome	*Vasospasm:* IL-1ra correlated to DIND ($p = 0.04$) IL-1ra peaked ~day 6 post-bleed and then decreased in good grade patients, while it remained elevated in poor grade patients	CSF IL-1ra, sTNFR, IL-6 may be associated with poor outcome at 6 months
Höllig et al. (21)	EVD-based sampling At day 1 only	*IL-6, LIF, E-selectin, ICAM-1*	Not specified 18 patients clipped	*Outcome assessed via dichotomized mRS (good = 0–2; poor = 3–6)* *Outcome at Discharge:* CSF LIF was associated with discharge outcome *Outcome at 6 months:* None of the measured cytokines were associated with outcome	N/A	CSF LIF at day 1 post-admission may be associated with outcome a discharge
Mathiesen et al. (22)	EVD-based sampling (control group had banked LP CSF) Unclear sampling intervals	*IL-1a, IL-1b, IL-1ra, TNF-a*	Not specified Unclear clip vs. coil numbers	*Outcome assessed via dichotomized GOS at unspecified interval (good = 4 or 5; poor = 1–3)* Elevated CSF IL-1ra ($p < 0.05$) and TNF-a ($p < 0.05$) at days 3–11 were associated with poor outcome	*Vasospasm:* CSF IL-1ra was elevated in all patient with DIND ($n = 3$) *Controls:* All CSF cytokines were elevated compared to control samples	CSF IL-1ra and TNF-a measured at day ~3–11 post-bleed may be associated with outcome
Nakahara et al. (23)	EVD-based sampling Day 3, 7, and 14 post-admission	*HMGB-1, IL-6, IL-8, TNF-a*	Not specified All underwent clipping	*Outcome assessed via dichotomized GOS at 3 months (good = 4 or 5; poor = 1–3)* CSF HMGB-1, IL-6, IL-8, and TNF-a were elevated in the poor outcome group	N/A	CSF HMGB-1, IL-6, IL-8, and TNF-a may be associated with outcome at 3 months
Niwa et al. (24)	EVD-based sampling Daily for 14 days	*IL-6, MCP-1, IL-10, MIG*	Not specified All underwent clipping	*Outcome assessed via dichotomized GOS at 3 months (good = 4 or 5; poor = 1–3)* Peak IL-6 was associated with poor outcome	N/A	CSF IL-6 may be associated with outcome at 3 months
Provencio et al. (25)	EVD-based sampling Daily for first 3 days	*IL-1a, IL-1ra, IL-2, IL-8, IL-17, TNF-a, INF-g*	Not specified Unclear clip vs. coil numbers	*Outcome assessed via dichotomized mRS at unspecified interval (good = 1–2; poor = 3–5)* Elevated CSF levels of IL-1a, IL-1ra, IL-2, IL-8, IL-17, TNF-a, and INF-g were found in the poor outcome group (all $p < 0.05$)	*N/A*	CSF IL-1a, IL-1ra, IL-2, IL-8, IL-17, TNF-a, and INF-g may be associated with outcome at 3 months
Sokół et al. (26)	EVD-based sampling (control group—non-ill patients with banked LP CSF) Day 1, 5, and 10 post-bleed	*HMGB-1*	Not specified All coiled	*Outcome assessed via dichotomized GOS at 3 months (good = 4 or 5; poor = 1–3)* CSF HMGB-1 levels were elevated at all 3 time points in those with poor outcome. Levels above 10 ng/mL were found in all with poor outcomes	*Controls:* SAH patients had higher HMGB-1 levels compared to controls	CSF HMGB-1 may be associated with poor outcome

(*Continued*)

TABLE 4 | Continued

Reference	Interval of cytokine measure	CSF cytokines measured	Interventional therapies applied during measurement	Outcome of interest	Other interesting CSF cytokine-related outcomes	Conclusions
Wada et al. (27)	LD-based sampling Day 1, 3, 6, and 9 post-admission	*G-CSF*	Not specified 8 clipped	*Outcome assessed via mortality at unspecified interval* Day 1 elevated G-CSF levels were associated with mortality	*Vasospasm:* No correlation between CSF G-CSF levels and vasospasm	CSF G-CSF levels may be associated with mortality
SHUNT DEPENDENCY STUDIES						
Douglas et al. (28)	EVD-based sampling Q2 day sample intervals (control samples collected from 7 patients with non-hemorrhagic communicating hydrocephalus)	*TGF-b1, TGF-b2*	Not specified Unclear clipping vs. coiling numbers	*Hydrocephalus as measured via F/U CT at 2 months:* CSF total TGF levels were higher in those patients whom developed CT-based hydrocephalus ($p < 0.05$)	*Controls comparison:* CSF TGF levels were higher in aSAH patients vs. controls	CSF TGF levels within the acute phase post-aSAH may predict chronic communicating hydrocephalus
Kitazawa and Tada (29)	Cisternal CSF or LD sampling Unclear sampling interval up to day 17	*TGF-b1*	Not specified 23 clipped	*Shunt dependency at 3 months:* No relation between CSF TGF-b1 and CT based peri-ventricular Hounsfield units CSF TGF-b1 on days 9–17 were higher in those whom developed ventricular dilatation on CT ($p < 0.02$) and VPS dependency ($p < 0.02$)	N/A	CSF TGF-b1 levels during the second week post-aSAH may be associated with the development of ventriculomegaly and VPS dependency
Takizawa et al. (30)	LP at day 14 post-bleed Control samples collected *via* LP	*IL-1b, IL-6, TGF-b1*	Not specified Unclear coil vs. clip numbers	*Shunt dependency at unspecified interval:* TGF-b1 levels were higher in those requiring a VPS	*Control comparison:* CSF levels of all cytokines were higher in the aSAH group	CSF TGF-b1 levels at 2 weeks post-bleed may be associated with shunt dependency
Wostrack et al. (31)	EVD-based sampling Q2 days for 14 days	*IL-6*	Not specified Unclear coil vs. clip numbers	*Shunt dependency at unspecified interval:* CSF IL-6 > 10,000 pg/mL was associated with VPS dependency ($p = 0.009$)	*N/A*	CSF IL-6 levels may be associated with VPS dependency
NIL ASSOCIATION STUDIES						
Gaetani et al. (32)	Cisternal CSF gathered at surgery	*IL-6, IL-8, MCP-1, E-selectin*	Not specified All were clipped	*Vasospasm:* No association between measured CSF cytokines and development of vasospasm (TCD MCA > 160 cm/s)	N/A	CSF IL-6, IL-8, MCP-1, E-selectin are not associated with vasospasm
Kaestner and Dimitriou (33)	EVD-based sampling Daily for 10 days	*TGF-b1, TGF-b2*	Not specified Unclear coil vs. clip numbers	*Chronic hydrocephalus (defined on CT and need for VPS; followed for 6 months post-bleed):* No correlation between CSF TGF levels with hydrocephalus and VPS dependency	*N/A*	CSF TGF-b1 and TGF-b2 levels are not associated with post-aSAH hydrocephalus or VPS dependency
Kim et al. (34)	EVD or LD sampling Daily up to day 14	*MIP-1*	Not specified Unclear clip vs. coil numbers	*Outcome assessed via dichotomized mRS (good = 0–3; poor = 4–6) at discharge:* CSF MIP-1 was not predictive of outcome	*Vasospasm:* CSF MIP-1 provides unclear prediction of vasospasm post-aSAH	CSF MIP-1 does not predict discharge outcome or vasospasm
Kwon and Jeon (35)	EVD-based sampling Unclear intervals	*IL-1b, IL-6, TNF-a*	"Triple H therapy"; not otherwise specified Unclear clip vs. coil numbers	*Outcome assessed via dichotomized GOS at unspecified interval (good = 4 or 5; poor = 1–3)* None of the measured cytokines were associated with outcome	*Vasospasm:* CSF IL-6 levels were higher in the DIND group ($p < 0.05$)	CSF IL-1b, IL-6, and TBF-a do not correlate with outcome at 6 months

(*Continued*)

TABLE 4 | Continued

Reference	Interval of cytokine measure	CSF cytokines measured	Interventional therapies applied during measurement	Outcome of interest	Other interesting CSF cytokine-related outcomes	Conclusions
Shoch et al. (36)	EVD-based sampling	*IL-6*	Not specified	*Vasospasm (as assessed via TCD):*	N/A	CSF IL-6 is not associated with patient outcome
	Daily for 14 days		65% treated *via* coiling	Elevated peak CSF IL-6 on day 6 post-bleed was associated with TCD-defined vasospasm DIND was associated with day 7 CSF IL-6 ($p = 0.03$) *Outcome as assessed by dichotomized mRS at unspecified interval (good = 0–2; poor = 3–6)* No association between IL-6 and patient outcome		CSF IL-6 may predict TCD vasospasm and subsequent DIND
Singh et al. (37)	EVD-based sampling	*IL-1ra, IL-1a, IL-1b, IL-6, IL-8, IL-10, MCP-1, TNF-a*	Randomized to standard therapy ($n = 7$) or IV IL-1ra ($n = 6$)	*Outcome as assessed by GOS at 6 months:*	IV IL-1ra lead to a decrease in CSF IL-6 from 6 to 24 h post-bleed, compared to placebo group	CSF cytokines are not associated with patient outcome (note: studied underpowered = acknowledged in manuscript)
	Q6 hours for 24 h post infusion of IL-1ra		Unclear clip vs. coil numbers	No association between CSF cytokine factors and outcome (i.e., Decreased CSF cytokine levels with IL-1ra were not associated with outcome)		

aSAH, aneurysmal subarachnoid hemorrhage; mRS, modified Rankin scale; GOS, Glasgow outcome scale; CMD, cerebral microdialysis; EVD, external ventricular drain; LP, lumbar puncture; VPS, ventriculoperitoneal shunt; ICP, intracranial pressure; CT, computed tomography; CSF, cerebrospinal fluid; LPR, lactate:pyruvate ratio; DC, decompressive craniectomy; IL, interleukin; a, alpha; b, beta; g, gamma; TNF, tumor necrosis factor; INF, interferon; MCP, monocyte chemoattractant protein; MIP, macrophage inflammatory proteins; TGF, transforming growth factor; EGF, epidermal growth factor; TNFR, tumor necrosis factor receptor; GM-CSF, granulocyte macrophage colony-stimulating factor; HMGB, high-mobility group box; DIND, delayed ischemic neurological deficit; CPP, cerebral perfusion pressure; TCD, transcranial Doppler; sTNFR, soluble tumor necrosis factor receptor; PDGF, platelet-derived growth factor; MCA, middle cerebral artery.

(10–18). There were six prospective studies, with all being prospective observational studies (10, 11, 13–15, 18). Three studies were retrospective case series (12, 16, 17).

A total of 246 unique patients with SAH were described across the 9 studies included in the CMD cytokine review. Two studies reported the same group of 88 SAH CMD patients, with a focus on analyzing different cytokine measures (15, 16). We took this into account during the calculation of the total patient numbers, to avoid counting patients twice.

The patient populations described within the CMD cytokine manuscripts were heterogeneous collections of aneurysmal SAH. The majority of studies focused on patients with poor admission clinical grades, classified as Hunt and Hess (H + H) grade 3–5, or as World Federation of Neurological Surgeons (WFNS) grade 3–5; often with high Fisher CT grade hemorrhages (i.e., 3 or 4). Aneurysm locations varied between studies, with some included both anterior and posterior circulation aneurysms. When recorded, the highest percentage of aneurysms was located within the anterior circulation with: anterior communicating artery (AComm), middle cerebral artery (MCA), and posterior communicating artery (PComm) locations predominating. Three studies failed to disclose patient clinical grade, radiographic grade, and aneurysm location information (14–16). Therapies received in the ICU were not clearly specified in the majority of the studies. Similarly, aneurysm treatment technique varied between and within the individual studies, with microsurgical clipping predominating.

We believe that some of the studies included within this portion of the review may contain duplicate patient information, as marked in **Tables 1** and **3**. Multiple publications from the same research groups likely were conducted on the same patient populations, yielding unique and separate manuscripts on the same group of patients. However, it is important to acknowledge that it was difficult to determine, in some circumstances, whether CMD cytokine analysis was being conducted on new patient groups or existing banked samples from previous prospective studies. With that said, our goal for the CMD cytokine scoping review was to provide an overview of all available literature in the area; hence, we have included all published papers on CMD cytokines in aneurysmal SAH within this review.

CSF Cytokine Review

Of the 20 articles included in the CSF cytokine systematic review (10, 19–37), 17 were formal manuscript publications (10, 20–24, 26, 28–37) and 3 were meeting abstract publications (19, 25, 27). All were prospective studies, with 19 being observational studies (10, 19–36) and 1 being a randomized control trial (37).

The populations described with in the CSF cytokine studies were quite heterogeneous, similar to the CMD cytokine papers. Most studies focused on patients with poor clinical grade (i.e., H + H 3–5; WFNS 3–5), and high Fisher CT grade (i.e., 3 or 4) upon admission. Aneurysm location varied significantly between papers with both anterior and posterior circulation aneurysms

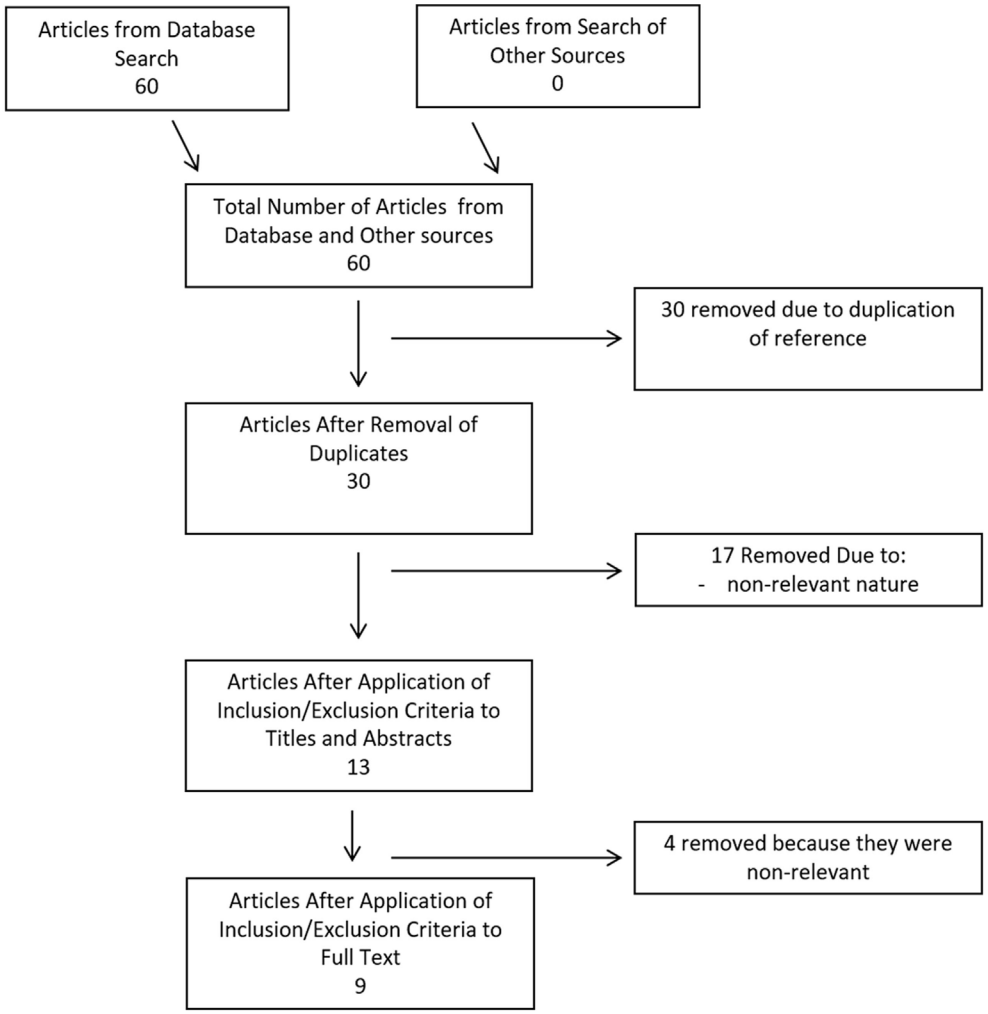

FIGURE 1 | Flow diagram of search results for cerebral microdialysate cytokines.

included in the studies. The majority of patients had anterior circulation aneurysms with AComm, MCA, and PComm representing the three most common locations. Therapies received within the ICU were either not specified or minimally characterized, leading to potentially significant treatment heterogeneity during CSF cytokine measurement. Finally, aneurysm treatment technique was unspecified in many studies (10, 19, 22, 25, 28, 30, 31, 33–35, 37). Those studies which disclosed aneurysm treatment employed both coiling and microsurgical clipping (20, 21, 23, 24, 26, 27, 29, 32, 36).

A total of 630 patients were described across all studies included in the CSF cytokine systematic review. The mean age for each study cohort varied significantly across studies, with all studies focusing on adult aneurysmal SAH. Details surrounding patient cohort, study design, and concurrent therapies can be found in **Tables 2** and **4**.

We made substantial efforts to exclude duplicate patient data across studies. However, given that many of the papers came from centers of excellence for TBI research, some of the patient data may be cross reported in multiple studies. This would reduce the total overall number of unique patients slightly. It was impossible based on the information provided within the parent studies to tease out all patients, which were reported more than once.

Cytokine Measurement Technique

CMD Cytokine Review

Location of the CMD catheter varied significantly between studies and was the following: mixed healthy/peri-lesional tissue in six studies (13–16, 18), territory of the aneurysm in two studies (10, 17), and unknown tissue location in two studies (11, 13). Some studies utilized paired microdialysis catheters, one in healthy and one in peri-lesional tissue (15, 16).

Analysis interval for CMD samples was as follows: every 6 h in five studies (11, 12, 14–16), every 8–12 h in one study (17), and unspecified in three studies (10, 13, 18). The duration of sample collection varied as well, with the typical collection period of 36 h to 10 days.

Numerous different panels of cytokines were evaluated within the CMD samples, across the studies included within the review. The most commonly studied cytokines included IL-1b, IL-6,

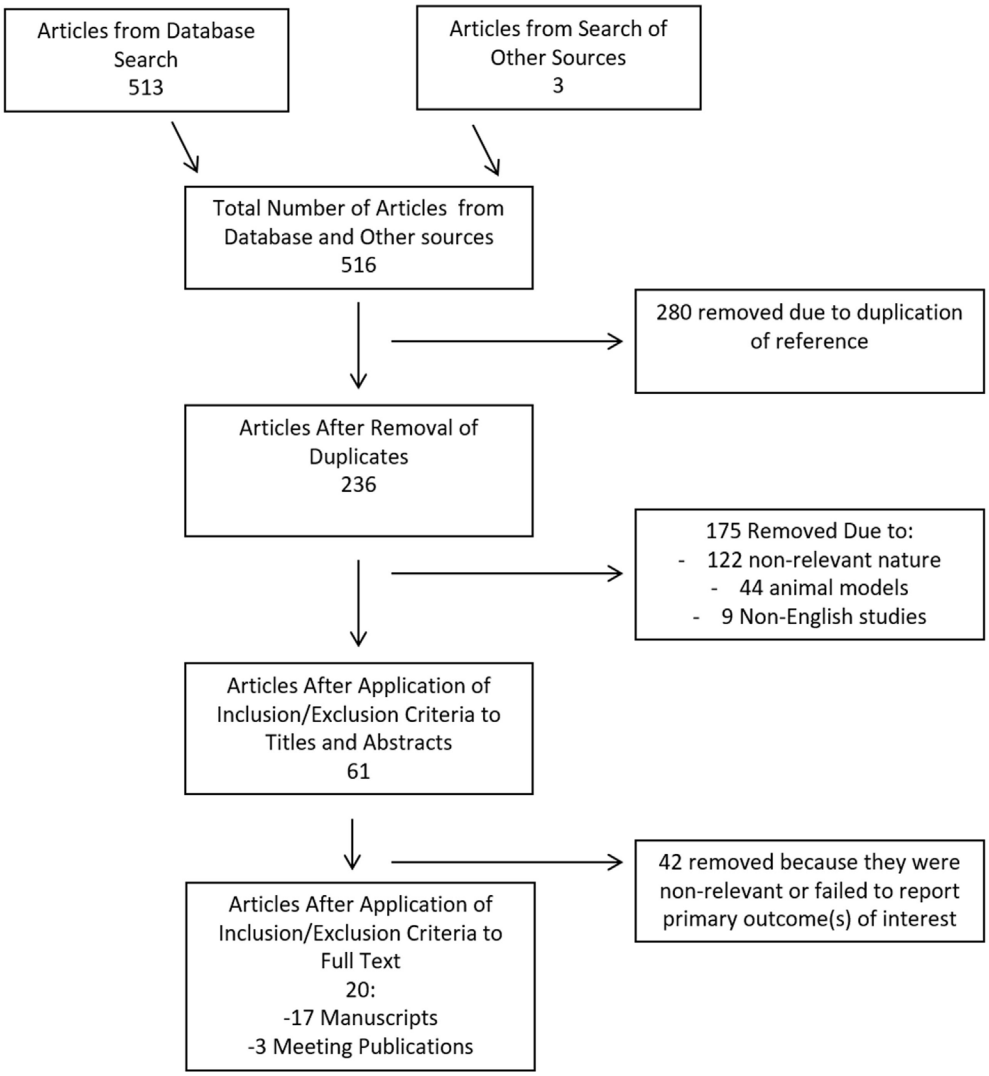

FIGURE 2 | Flow diagram of search results for cerebrospinal fluid cytokines.

IL-10, and TNF-a. Details surrounding CMD technique and catheter locations can be seen in **Table 3**.

CSF Cytokine Review

Sampling of CSF was conducted through external ventricular drains (10, 19–26, 28, 35–37), cisternal collection intraoperatively (29, 32), or by lumbar puncture (27, 29, 30, 34). Sampling and analysis frequency varied significantly from study to study with sampling occurring from daily to every 2–3 days. Duration of sampling varied as well, up to a maximum of 14 days post-ictus (31).

Like the CMD cytokine papers, the CSF cytokine papers included in this review reported the measurement of various cytokines. The most commonly measured cytokines in CSF reported were IL-1b, IL-1ra, IL-6, IL-8, TNF, TGF, and MIP. The details of CSF sampling and specific cytokines measured can be found in **Table 4**.

Outcomes

CMD Cytokine Review

Given that the CMD cytokine portion of this review was a scoping review, providing an overview of all the available literature on CMD cytokine measures in aneurysmal SAH, the outcomes reported by the studies are quite heterogenous. They can be seen in detail in **Table 3**.

Three studies reported the correlation between CMD cytokines and patient outcome (10, 13, 18). All of these three studies reported a correlation between elevated CMD IL-6 levels and poor outcome at 6 months, measured using the Glasgow Outcome Scale (GOS) (10), 3 months measured using the modified Rankin scale (mRS) ($p = 0.01$) (13), or other unspecified outcome scales ($p < 0.01$) (18).

Both the presence of intraventricular hemorrhage and intracerebral hemorrhage (ICH) post-aneurysm rupture were associated with elevated CMD IL-6 ($p = 0.003$) (18) and TNF-a (11).

Similarly, peri-lesional probe CMD probe location was associated with higher IL-6 levels compared to more distant probe locations ($p = 0.002$) (18).

Radiographic cerebral vasospasm was found to be associated with elevated CMD TNF-a on day 4 and 6 post-ictus in one study ($p < 0.01$) (12). Similarly, elevated total CMD IL-6 levels were found to be associated with radiographic vasospasm in one study (17). However, there was an unclear association with the development of DIND.

Many studies provided descriptions of CMD cytokine profiles and temporal patterns. Given the various cytokines measured across the studies, it is impossible to describe all of these relationships, but highlights from these analyses are presented in **Table 3**. Broadly speaking, the data show temporal variations in cytokine levels, with peaks in IL-1b, IL-6, IL-8, and MIP between 6 and 12 h post-bleed (14, 15). On the other hand, IL-10 levels in CMD remained constantly elevated throughout the analysis periods recorded (14, 15).

CSF Cytokine Review

Within the 20 papers included in the CSF systematic review (10, 19–37), we found both manuscripts which reported positive associations between CSF cytokines with patient outcome/chronic hydrocephalus/VPS dependency (10, 19–31) and studies reporting no association (32–37) (i.e., "nil association") between CSF cytokines and the outcomes of interest for the CSF cytokine systematic review. No studies identified reported association, "nil" or otherwise, between CSF cytokine measures and tissue outcome as assessed by follow-up neuroimaging. The subsections below describe more details around these outcomes of interest, with further information found in **Table 4**.

Positive Association Studies

Fourteen papers included within the CSF cytokine review found associations between measured cytokines with both patient outcome and/or chronic hydrocephalus/VPS dependency. Ten of these reported an association between CSF cytokines and patient outcome (10, 19–27). Four papers reported an association between CSF cytokine measures and the development of chronic hydrocephalus/VPS dependency (28–31).

Patient Outcome. The strongest relationships between CSF cytokine levels and clinical outcome (defined using the GOS or mRS) were seen for IL-6 (10, 20, 23, 24), IL-1ra (20, 22, 25), IL-8 (23, 25), and TNF-a/sTNFR (20, 22, 23, 25). Associations between CSF levels of high-mobility group box-1 (26), G-CSF (27), LIF (21), and IL-1a (25) and poor patient outcome were also described.

Chronic Hydrocephalus/VPS Dependency. Four studies discussed the correlation between CSF cytokines and the development of chronic hydrocephalus/VPS dependency (28–31). One study showed that CSF TGF-b1 and TGF-b2 levels were associated with the development of hydrocephalus (defined using CT imaging) at 2 months post-bleed ($p < 0.05$) (28). A second study also documented the correlation between elevated CSF TGF-b1 during the patients ICU stay and the development of radiographic hydrocephalus or VPS dependency at 3 months ($p < 0.02$) (29). A third study confirmed that CSF TGF-b1 levels were elevated during the acute/subacute phase in those who became shunt dependent (30). Finally, one study documented that CSF IL-6 levels during the acute/subacute phase post-bleed to be associated with VPS dependency at an unclear interval ($p = 0.009$) (31).

Nil Association Studies

Our review identified six studies documenting a "nil association" between CSF measured cytokines in aneurysmal SAH patients and various outcomes of interest (32–37).

Four studies reported no association between various CSF cytokines and patient outcome, as reported by in-hospital mortality or GOS at 3–6 months (34–37). The cytokines reported within these studies varied significantly, with the most common "nil associations" reported for MIP (34), IL-1b (35, 37), IL-6 (35, 37), and TNF-a (35, 37). A total of 140 patients were described within these studies, compared to the 283 patients within the studies documenting a correlation between CSF measured cytokines and patient outcome (10, 18–27).

Two studies reported no association between CSF cytokine measures and TCD-based flow velocity (32, 36), while one study failed to show an association between CSF TGF and VPS dependency (33). Further detail on the "nil association" studies can be found at the bottom of **Table 4**.

Complications

Within the CMD cytokine manuscripts, all manuscripts failed to report whether complications were considered within the data collection. We suspect that complication profiles are dramatically underreported within the CMD studies.

Complication reporting within the CSF cytokine studies was essentially non-existent, with the focus of these studies being the association between CSF cytokine measures and various outcomes.

DISCUSSION

CMD Cytokines in SAH

The scoping systematic review on CMD cytokines in aneurysmal SAH yielded nine studies. Despite the small number of studies and patients described within, there are a few points of interest that deserve highlighting. First, CMD-based measurement of cytokines is feasible in this patient population. Second, CMD catheter location makes a difference in the levels of cytokines measured, with peri-lesional tissue producing high levels compared to distant or healthier tissue (18). Third, peaks in CMD cytokine measures may occur within the first 6–12 h for IL-1b, IL-6, IL-8 and, MIP, while IL-10 seems to remain elevated in CMD samples through the duration of the sampling periods described (14, 15). Fourth, CMD IL-6 levels may be associated with poor outcome (10, 13, 18), up to 6 months post-injury. Finally, complications related to the use of CMD catheters are underreported, and there is a concern of selective harms reporting within the literature identified.

CSF Cytokines in SAH

The systematic review on CSF cytokines in aneurysmal SAH, focused on the association between cytokine measures with patient outcome, chronic hydrocephalus/VSP dependency, neurophysiologic outcome, or tissue outcome. We identified some interesting trends from the 20 included studies (10, 19–37). First, a broad range of cytokines or panels of cytokines were described in these studies, but the strongest associations with poor outcome were found for elevated CSF levels of: IL-6 (10, 20, 23, 24), IL-1ra (20, 22, 25), IL-8 (23, 25), and TNF-a/sTNFR (20, 22, 23, 25). Second, acute/subacute CSF levels of TGF-b1 and TGF-b2 seemed to be associated with chronic hydrocephalus or shunt dependency at 2–3 months post-bleed (28–30). Third, we were unable to identify any studies documenting an association between CSF cytokines measures in SAH with neurophysiologic or tissue-based outcomes. Fourth, despite the "positive" associations found in the previously described papers, four manuscripts found no relationship between CSF cytokines and patient outcome (34–37). The patient numbers within these studies were smaller than that in the studies describing a positive association between CSF cytokines and patient outcome, with the "nil association" studies totaling 140 patients and the "positive association" studies totaling 286 patients. Finally, the complication reporting within the CSF cytokine studies was absent. Selective reporting bias here is a major concern.

Limitations

Despite the interesting results of these two systematic reviews, there are significant study limitations that need to be highlighted. Limitations with each separate review can be found within the subsections to follow. Two limitations affected both reviews.

First, which was eluded to within the Methods section, is the inclusion of meeting abstracts. This could be considered controversial; however, to provide the most comprehensive scoping systematic review on this relatively "new" field of research in SAH, we thought it necessary to include these studies. Furthermore, many negative studies are presented at meeting venues, never reaching manuscript form. We wished to include any of these potential negative result abstracts to reduce publication bias seen within only positive studies. Yet, one must be cautioned in overinterpreting the results of the meeting abstracts. Given the nature of these publications, the quality of evidence is low and they are subject to significant reporting biases.

Second, within both the CMD and CSF reviews, some studies had missing data points, as seen within the tables. We made two distinct and separate attempts to contact the authors for information regarding these studies (i.e., missing demographics, etc.). The first was made in November 2016, with a second attempt in January 2017. Both were met with no response *via* electronic communication. Thus, we were unfortunately left with leaving these fields as "unknown" or "uncertain" within the tables. Although this is unfortunate, as the overall picture for each study may not be complete, this tends to be the nature of systematically conducted reviews within new and emerging areas of research.

Finally, the exact details on the cytokine measurements were not clearly delineated in most studies. With little comment on what was done to reduce interassay variability, as this could contribute to conflicting results seen within the review. Some mentioned use an enzyme-linked immunosorbent assay (ELISA) for the cytokine(s) of interest, while others mentioned multiplex "plates" for and array of cytokines, without further details. Furthermore, the timing of cytokine measurement was not mentioned or taken into consideration the reported studies. Thus, there is potential for normal circadian variation in cytokine profiles to have impacted the results reported.

CMD Cytokine Review

First, there were a small number of heterogenous studies found for the CMD review, with some manuscripts reporting on the same patient populations based on banked CMD samples. Furthermore, all included studies described heterogeneous cohorts of aneurysmal SAH patients with varying clinical/radiographic admission grades and aneurysm locations, making summary interpretation of results difficult. Second, the ICU and surgical therapies received by these patients during CMD sample collection/processing was quite heterogeneous. Many studies failed to specify the therapies or protocols initialized within the ICU. These treatment variations may lead to substantial changes within the CMD cytokine measures. Thus, the described associations or "nil associations" may not be accurate given this potential confounder. Third, across all the studies, there was variation in CMD catheter location. This could impact the CMD cytokine measures obtained and the described relationships. In addition, the CMD perfusate, sampling frequency, use of pooled analysis, and cytokine panel/analytic platform employed varied between studies. Given this, it is impossible for us to directly compare the absolute values of cytokines and relative recovery. Thus, our reporting of the results for CMD in SAH is limited to purely descriptive. Fourth, complications associated with CMD monitoring were seldom reported. Given the total number of patients studied, it is unlikely that there were no patients suffering from complications of invasive monitoring. Finally, given the studies and results identified for the CMD review, there is likely a large publication bias, favoring only studies with positive results.

CSF Cytokine Review

First, there were many quite heterogeneous studies identified in the CSF cytokine review. The included papers varied by number of patients, admission clinical/radiographic grades, aneurysm location, aneurysm treatments (clipping vs. coiling), surgical interventions, ICU-based therapies offered/provided to patients, blinding during outcome assessment, primary outcome of the studies, and duration of follow-up. These limitations suggest caution when interpreting or generalizing the results of studies that describe relationships between CSF cytokine measures and patient outcomes. Second, many cytokine associations were selectively reported, making no reference to other CSF measures and the results of statistical analysis. Therefore, there may be many more "nil associations" that were not disclosed within the body of the manuscripts. Third, complication reporting was concerning within the literature identified (as mentioned above), with underreporting is suspected. Fourth, given all the above limitations and

heterogeneity issues, a meta-analysis was not performed. Finally, given the overwhelming number of "positive association" studies identified, the literature likely suffers from significant publication bias.

CMD Technical Considerations

The complexity involved in cytokine retrieval from CMD requires some brief comments regarding some potentially more "standardized" techniques. First, standard CMD catheters employ pore sizes between 20 and 100 kDa, as the goal with these devices is to measure "common" analytes such as glucose, glutamate, glycerol, lactate, and pyruvate. Although well designed for this purpose, they are ineffective for the retrieval or larger protein biomarkers, such as cytokines, where molecular weight can easily exceed these pore size. This it is critical to know the characteristics of the biomarker of interest, thus tailoring your CMD catheter to the biomarker (44). Second, the location of placement is key. Within the TBI literature, it has been well documented that CMD catheter placement in lesional vs. peri-lesional tissue yields very different profiles of "common" analyte retrieval (45). This has also been demonstrated within CMD cytokine profiles in TBI, with lesional/peri-lesional tissue expressing much high cytokine levels compared to healthy tissue (46, 47). Thus, we recommend placement of the CMD catheter within the brain adjacent to a focal lesion or territory of interest. This way, the "at risk" brain would be monitored and not the irreversibly damaged areas. Third, the rate of perfusion should remain at 0.3μl/min. Higher perfusion rates may impair the rate of uptake of these larger proteins (44), while there is not data to support improved recovery for lower rates. Fifth, the perfusate should be colloid based. Recent investigation into the type of perfusate has demonstrated that the relative rate of recovery for cytokines is improved with colloid perfusate over crystalloid (44). The exact colloid solution to use is currently unclear. Albumin solution appears to improve the relative recovery (44); however it is expensive and labor intensive to create, thus limiting its widespread applicability. Dextran-based solutions are another potential and have been applied within some of the SAH studies quoted within this review (14, 16). However, the literature surrounding the type of dextran solution to use is limited, and we cannot make any further definitive comments at this time. Sixth, it is unclear at the current time as to the impact of frequency of CMD measurement and pooling of samples. Given we do not currently have a clear idea of the temporal profile of cytokine changes in CMD fluid, we cannot give definitive recommendations regarding the sampling frequency. Although, we would expect the rate of change in focal cytokine profile be on the order of hours or longer (such as 6 to 12 h). Finally, the cytokine analysis technique requires some comment and is applicable to both CMD and CSF analysis. Both ELISA and multiplex-based techniques have been described. All techniques are subject to inter-assay variability and thus should be conducted within established laboratory settings with trained personal, comfortable with the employed technique. The use of ELISA vs. multiplex may be site dependent or study specific. Standard analytic techniques should be employed for multicenter collaborations to improved homogeneity in measurement technique. This also applied to the entire process of CMD or CSF sampling, storage, and analysis. In addition, the normal variation in cytokine profiles should be taken into account when determining sampling frequency and pooling samples for analysis. It is particularly important to ensure that between patients, the sampling frequency and employment of sample pooling is conducted in an identical manner. Circadian variation in cytokine profiles (48) could impact the interpretation of results, and thus, standardized sampling and pooling is a necessity.

Future Directions

Given the limited literature body and the recognized limitations in study design, there exists an opportunity for further research in the area of CMD and CSF cytokines in aneurysmal SAH, but these should seek to address the limitations seen in the studies included within the two systematic reviews. First, larger cohorts of aneurysmal SAH patients with predefined stratification of injury pattern are required. Heterogeneity in hemorrhage pattern, clinical grade, and aneurysm location make the results of the above-mentioned studies difficult to interpret, even those with positive results. Large sample sizes may allow for clinical/radiographic subgroup analysis and shed further light on the association of CMD/CSF cytokines with various subpopulations of aneurysmal SAH patients. While large studies undertaken with a uniform protocol would be ideal, we need to accept that these studies will often be conducted in relatively small populations of patients across several centers. Such a multiplicity of studies could be a substantial strength in exploring the pathophysiology and outcome associations of central nervous system cytokine levels across the spectrum of aSAH if we could undertake harmonization of the studies. Consistency across multiple centers would require rigorous harmonization of studies, which would only be possible if there were clear data provided on disease characteristics, catheter location, sample processing, and measurement techniques; and all studies used a common outcome assessment (e.g., GOSE at 3 months). With the application of common data elements between studies and centers, we may be able to more closely approach harmonization. Furthermore, banking of CSF and CMD samples from various SAH studies could prove to be a useful way of increasing the sample numbers required to analyze the milieu of CSF/CMD based cytokines. Second, homogeneous ICU/surgical treatments are necessary, preferably with protocolized therapies. Including coiled and clipped patients within the same cohort of SAH patients assuredly confounds the associations between various measured cytokines and the described outcomes. In addition, including patients with ICH evacuation and those undergoing DC due to malignant edema will also impact the resulting of cytokine measures. Third, with the application of CMD, catheter location must be considered during cytokine measures. Fourth, given the large number of cytokines involved, the use of principle component analysis of large patient populations with CMD and CSF cytokine measures may prove valuable. This has been applied within the TBI literature on CMD cytokines, with interesting preliminary results (49, 50). This could potentially identify cytokine patterns of co-expression in CMD and CSF, highlighting targets for future studies and therapeutic intervention. Fifth, accurate complication documentation is required. Sixth, one persistent problem may be the use of different analysis

platforms, which results in different measured concentrations. There are no easy solutions to this problem—although control plasma levels will provide some basis for harmonization, it will be difficult to get standardize levels in CSF and particularly in CMD. Finally, multicenter prospective evaluation of cytokines within CMD and CSF is necessary to improve patient recruitment and aid with spreading the substantial cost of cytokine analysis among centers. Without collaboration, single-center small studies may unfortunately fail to add to the existing literature.

CONCLUSION

The evaluation of CMD and CSF cytokines is a new area of the literature in aneurysmal SAH. The two scoping systematic reviews demonstrated the following: (1) limited literature available on CMD cytokine measurement in aneurysmal SAH with some preliminary data supporting feasibility of measurement and potential association between IL-6 and patient outcome. (2) CSF levels of several cytokines may be associated with patient outcome at 3–6 months including IL-1ra, IL-6, IL-8, and TNF-a. (3) There is a small literature supporting an association between acute/subacute CSF TGF levels and the development of chronic hydrocephalus at 2–3 months. Given the preliminary nature of these data, further large prospective multicenter studies on cytokines in CMD and CSF need to be conducted.

AUTHOR CONTRIBUTIONS

FZ was responsible for concept, design, systematic review searches, data acquisition/extraction, data analysis, manuscript composition, and editing. ET was responsible for systematic review searches, data acquisition/extraction, data analysis, manuscript composition, and editing. MC was responsible for data analysis, manuscript composition, and editing. PH was responsible for data analysis and manuscript composition/editing. DM and AH were responsible for concept, data analysis, and manuscript composition/editing.

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REVIEW
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Monitoring the Neuroinflammatory Response Following Acute Brain Injury

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Traumatic brain injury (TBI) and subarachnoid hemorrhage (SAH) are major contributors to morbidity and mortality. Following the initial insult, patients may deteriorate due to secondary brain damage. The underlying molecular and cellular cascades incorporate components of the innate immune system. There are different approaches to assess and monitor cerebral inflammation in the neuro intensive care unit. The aim of this narrative review is to describe techniques to monitor inflammatory activity in patients with TBI and SAH in the acute setting. The analysis of pro- and anti-inflammatory cytokines in compartments of the central nervous system (CNS), including the cerebrospinal fluid and the extracellular fluid, represent the most common approaches to monitor surrogate markers of cerebral inflammatory activity. Each of these compartments has a distinct biology that reflects local processes and the cross-talk between systemic and CNS inflammation. Cytokines have been correlated to outcomes as well as ongoing, secondary injury progression. Alongside the dynamic, focal assay of humoral mediators, imaging, through positron emission tomography, can provide a global *in vivo* measurement of inflammatory cell activity, which reveals long-lasting processes following the initial injury. Compared to the innate immune system activated acutely after brain injury, the adaptive immune system is likely to play a greater role in the chronic phase as evidenced by T-cell-mediated autoreactivity toward brain-specific proteins. The most difficult aspect of assessing neuroinflammation is to determine whether the processes monitored are harmful or beneficial to the brain as accumulating data indicate a dual role for these inflammatory cascades following injury. In summary, the inflammatory component of the complex injury cascade following brain injury may be monitored using different modalities. Using a multimodal monitoring approach can potentially aid in the development of therapeutics targeting different aspects of the inflammatory cascade and improve the outcome following TBI and SAH.

Keywords: neuroinflammation, traumatic brain injury, subarachnoid hemorrhage, multimodal monitoring, secondary brain injury

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INTRODUCTION

Pathophysiology of Brain Injury

Traumatic brain injury (TBI) and aneurysmal subarachnoid hemorrhage (SAH) are common neurological conditions (1, 2) associated with extensive morbidity and mortality (3, 4). While the two diseases are different entities, they share many common features in their secondary pathophysiology. Furthermore, they provide an experimental paradigm in which multimodality monitoring offers the opportunity to decipher common pathological processes. There are multiple potential mechanisms by which neuronal injury is inflicted following TBI and SAH. The traditional clinical paradigm for management of these conditions has focused on adequate delivery of oxygen and appropriate metabolic substrates to the injured brain, and prevention of secondary injuries caused by hypotension and hypoxia (5). These secondary processes lead to an increasingly inhospitable environment with ongoing excitotoxicity, oxidative stress, blood–brain barrier (BBB) disruption, cortical spreading depression, mitochondrial dysfunction, and subsequent cellular death in the tissue surrounding the initial damage (6, 7). There is an increasing recognition that inflammatory mediators are involved in the mechanistic link that underlies these injurious processes (8–10).

What Is Neuroinflammation?

Galen's original description of inflammation as *calor* (heat), *dolor* (pain), *rubor* (redness), *tumor* (swelling), and subsequently *functio laesa* (loss of function) has been refined into a more complex phenomenon that represents the host response to any insult. This complex process involves the release of molecular mediators, the alteration of the cerebral vasculature, the activation and influx of immune cells to eliminate pathogens, damaged cells, and other perceived harmful stimuli. The brain was long thought to be immunoprivileged due to the presence of the BBB, which limits the cross-talk between the blood and brain-resident inflammatory cells, the lack of a classical lymphatic system, and the shielding of neural antigens from peripheral immune surveillance. More recently, all these assumptions have been questioned and revised (11). There is an increasing realization that cerebral inflammation or neuroinflammation occurs within the whole gamut of central nervous system (CNS) pathologies, whether it is an adaptive autoimmune response (e.g., multiple sclerosis) or a response to external stimuli (e.g., accumulation of red blood cells following brain hemorrhage). While microglia are considered the primary immune cells of the CNS, it is becoming increasingly clear that other glial cells (astrocytes and oligodendrocytes) as well as neurons display immune-competent functions. Activated resident cells of the CNS, in combination with migrating inflammatory cells from peripheral blood, form an intricate immune network (12, 13). Although this immune response may be initiated to protect the brain, it is becoming evident that it can result in harmful outcomes for the CNS (14). In fact, an ongoing neuroinflammatory response may not only contribute to increased edema, cellular death, and BBB disruption but also function as a potent scavenger of dead cells and support the regenerative processes in the injured CNS (15, 16). An outline of the pathophysiological mechanisms identified in the literature is illustrated in **Figure 1**, many of which are described in this review.

Aspects of Neuroinflammation in SAH

Brain hemorrhage triggers a series of pathological processes resulting in neuronal damage and consequent neurological deficit (17–22). Early brain injury (EBI) refers to the damage ensuing in the first 72 h after the bleed caused by transient cerebral ischemia (CI), BBB disruption, edema, cell death, and brain tissue loss. Many of the patients who survive these phenomena deteriorate days later from delayed ischemic neurological deficit (DIND), which is responsible for poor outcomes or death in up to 30% of patients with SAH. DIND was traditionally attributed to narrowing of the large basal cerebral arteries, termed cerebral or angiographic vasospasm, causing a reduction in cerebral blood flow (CBF) and, if critically reduced, progressing to infarction in the relevant vascular territory. However, a growing number of reports shows that angiographic vasospasm does not always correlate with CI, cerebral infarction, or changes in CBF, indicating that DIND is a more complex (and complicated) phenomenon (23–33). The landmark CONSCIOUS-1 trial showed that an endothelin-1 antagonist ameliorates angiographic vasospasm, but fails to improve functional outcomes (34, 35), forever changing the concept of DIND, which is now suspected to arise from the combined effects of angiographic vasospasm, global reduction in blood flow, arteriolar constriction and thrombosis, cortical spreading depressions, and processes triggered by EBI (17–21, 36). With the complex interplay of the underlying pathological substrates, DIND is often (incorrectly) used as a blanket term for vascular spasm, CI, and neurological (clinical) deficit. It remains unclear to what extent these phenomena overlap or represent distinct processes (**Figure 2**).

It is increasingly recognized that the neuroinflammatory cascade following SAH is the potential mechanistic link between the initial ictus and the progression of neuronal injury in EBI as well as the development of DIND (37–40). Both overlapping phenomena of ischemic injury and vasospasm lead to an unfavorable physiological environment that may inflict neuronal injury (**Figure 2**), possibly driven in part by neuroinflammatory mediators.

Monitoring Neuroinflammation in the Neuro-Critical Care Unit (NCCU)

Patients suffering from TBI and SAH are typically treated in specialized NCCUs where extensive intracranial monitoring is implemented in order to detect subsequent secondary insults, prevent further deterioration, and optimize conditions for brain recovery (41, 42). Monitoring neuroinflammatory processes is technically and logistically complex, and this has hampered the development of pharmaceutical compounds that act to modulate the neuroinflammatory cascade (43). As we acquire a greater insight into the interplay between neuroinflammation and secondary brain injury, monitoring the neuroinflammatory events may open an opportunity to both increase our understanding of the molecular basis of the underlying pathology as well as identify novel therapeutic targets.

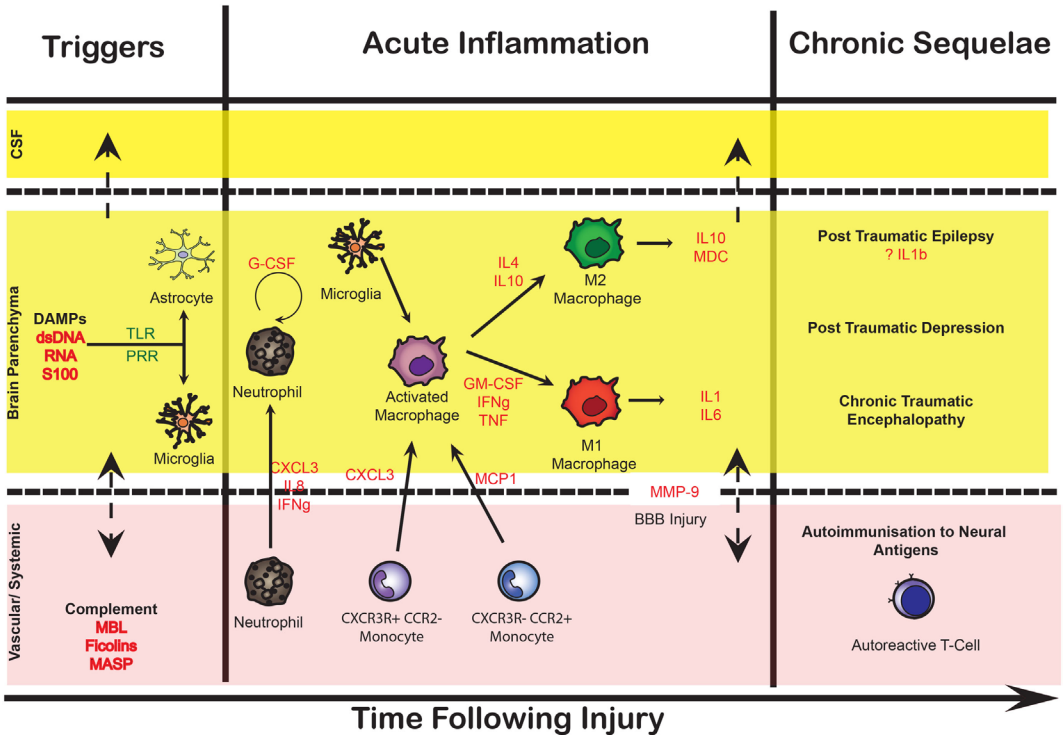

FIGURE 1 | Conceptual summary of the neuroinflammatory response in acute brain injury. This illustration depicts some of the cellular and molecular sequelae and inflammatory cascades that occur following injury to the brain over time, divided into different compartments [cerebrospinal fluid (CSF), brain parenchyma, and vascular]. Initially, danger-associated molecular patterns (DAMPs) including double-stranded (ds)DNA, RNA, and brain-enriched proteins such as S100B provide early triggers to resident cells reflecting tissue injury and linking the initial insult to subsequent humoral and cellular cascades. These in turn lead to an increasingly cytotoxic environment promoting cellular responses, stimulating both immunocompetent cells in the central nervous system (resident microglia and astrocytes), as well as recruiting peripheral monocytes, through pattern recognition receptors (PRR) such as toll-like receptors (TLR). The secretion of cytokines, chemokines, and other potent chemotactic substances (including complement proteins) induces the recruitment of neutrophils and monocytes into the brain parenchyma through the increasingly permeable blood–brain barrier (BBB), as well as having a direct cytotoxic effect. Ongoing release of cytokines initiates a long-term inflammatory process, which allows the dynamic shift of macrophages and microglial canonical phenotype between M1 (classical activation) and M2 (alternative active presumably the result of antigen-presenting cells migrating from the periphery). These processes result in chronic inflammation as well as auto-immunization toward brain-enriched antigens. Key cytokines are highlighted in red. MBL, mannose-binding lectin; MASP, mannose-associated serine protease; MMP-9, matrix metalloproteinase 9.

The aim of this narrative review is to demonstrate how different aspects of the neuroinflammatory response contribute to the pathophysiology of secondary brain injury. In addition, we discuss the methods available to monitor cellular and humoral inflammation in TBI and SAH patients in the clinical setting.

CYTOKINE AND CHEMOKINE MONITORING IN ACUTE BRAIN INJURY

There is no accepted gold standard in how to assess the neuroinflammatory processes in TBI and SAH. In clinical studies, the most common approach is to measure the concentration of cytokines and chemokines as biomarkers of ongoing neuroinflammation. Cytokines are small proteins of approximately 20 kDa molecular weight that are synthesized primarily by immune cells to promote and regulate inflammatory responses in an autocrine and paracrine fashion.

There are currently a wide range of commercially available assays that can detect with high sensitivity (pg/ml range) the levels of cytokines in different compartments. Historically, enzyme-linked immunosorbant assay has most frequently been used, but these require relatively large volumes of fluid/tissue and can only measure one cytokine at the time, and are therefore not ideal to monitor a complex inflammatory network. In the last 10 years, multiplex bead arrays have allowed the use of very small sample sizes (~25 µl per sample) to simultaneously measure several cytokines, thus greatly increasing the technical efficiency and our understanding of the inflammatory response in the injured brain (44).

CNS Compartmentalization of Cytokine Measurements

The CNS has several inherent barriers, restricting and controlling the flow of substances. These include the BBB, the blood–cerebrospinal fluid (CSF) barrier, and the CSF–brain barrier. The brain compartment, or specifically the extracellular space of the brain, is accessible by using a microdialysis (MD) catheter. The MD catheter possesses a semipermeable membrane that is able to extract proteins like cytokines directly from the extracellular fluid (ECF) when inserted into the injured brain (13, 45).

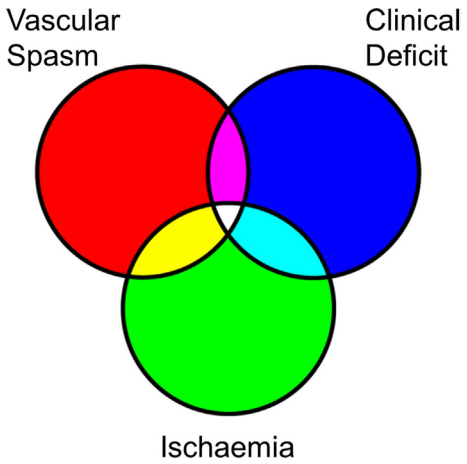

FIGURE 2 | Overlapping characteristics of the pathophysiology in subarachnoid hemorrhage (SAH). Simplified Venn diagram of the different pathophysiological processes that follow SAH. Rather than a linear relationship between vascular spasm, ischemia, and clinical deficit, these may be overlapping and also occur as distinct phenomena. Vascular spasm is most closely linked with endothelin-1-mediated vasoconstriction but may also encompass swelling of the vascular endothelium leading to an angiographic appearance of reduced contrast flow. Ischemia may result from an absolute reduction in blood flow, which may be complicated by local microvascular collapse or thrombosis, or even hyperemia causing localized swelling and reducing the capillary perfusion in that locality. Neurological deficits may be caused by frank ischemia and also result from phenomena such as cortical spreading depression.

The CSF is produced by cells of the choroid plexus located within the cerebral ventricles and provides a medium to embed the brain, maintain a homeostatic environment, and facilitate the transport of nutrients and waste products. Thus, while using the CSF for cytokine measurement may provide a global assessment of cerebral inflammation, it remains less specific in terms of regional monitoring. CSF is more easily accessible through, ideally, external ventricular drainage in sedated patients or *via* lumbar puncture in conscious patients where several milliliters can be drawn for analyses. While blood is biologically the most remote space from the organ of interest, it represents an easily accessible compartment for repeated sampling. To compare and monitor cytokine concentrations, collection of matched CSF and plasma samples over time allows a composite picture of ongoing immune responses, distinguishing central from systemic cytokine profiles and determining how immune activation in the brain may impact on mechanisms occurring in the periphery and *vice versa*.

Figure 1 illustrates the intricate relationship between different compartments in the CNS and the potential cross-talk between the different compartments.

Cytokines in Blood

Although controversial, some groups have correlated cytokine concentrations measured in blood/plasma in the acute and chronic setting of patients with TBI and SAH to functional outcome (46–48). This suggests that, while these mediators may represent non-brain-specific inflammatory activity, systemic inflammation is triggered by brain injury, potentially having long-term sequelae such as stimulating an acute-phase response in the liver (49). Importantly, the pattern of cytokine production is both qualitatively and quantitatively different in blood compared to CSF following injury (49–52), suggesting the existence of distinct biological processes at play.

Cytokines in CSF

Collection of CSF is the oldest and most prevalent method to test for inflammatory mediators following TBI and SAH and, and in contrast to blood sampling, it represents a global measure of cerebral immune activity. Access to ventricular CSF is achieved with surgical insertion of a drain in the ventricles to measure and monitor changes of intracranial pressure. Longitudinal measurement of cytokines in CSF of patients with severe TBI reveals a biphasic immune response being maximal in the first few days after injury followed by a second modest increase by 7–10 days as shown for IL-6 (49).

These data have been confirmed using principal component analysis (PCA) of CSF cytokines and chemokines, resulting in the identification of distinct clusters of cytokine response (53, 54). Interestingly, multivariate analysis has also demonstrated a significant difference in outcome between the two clusters. Thus, assessing inflammatory activity provides a potential window for appropriate pharmaceutical therapies targeting cytokine production (54). The difficulty is in disentangling the roles of individual mediators within these clusters to investigate rationalized treatment targets.

In SAH, CSF samples may not be a true measure of neuroinflammation. The volume of blood present within the subarachnoid space can affect the levels of cytokines present in CSF, and therefore, cytokine concentration in CSF is partly a consequence of the volume of SAH rather than a specific measure of the magnitude of the inflammatory response within the brain.

Microdialysate Monitoring of Brain ECF

Microdialysis is inevitably a spatially restricted approach that can be used to target pericontusional/infarcted tissue at risk for secondary injury and thus allow us to monitor ongoing or worsening focal brain damage. Notably, cytokine analysis revealed large variations in their concentrations when comparing CSF with brain-ECF (**Table 1**). However, the difference in cytokine levels between CSF and brain-ECF is smaller compared to the concentration gradients between these compartments and serum (55). Measurement of cytokines using MD have the additional complication of relative recovery. Relative recovery within the microdialysate fluid refers to the recovered fraction of the true concentration in the ECF, which is governed by a range of physicochemical factors including the molecular weight of the cytokine, its isoelectric point (which in turn determines the charge the protein carries at neutral pH and its solubility in water), and its native oligomerization characteristics (56). For some species, the relative recovery can be as low as 5% (exemplified in **Table 1**) making it technically difficult to reliably monitor.

There is an intricate interplay between the various cellular elements (both resident and recruited) within the brain, which

TABLE 1 | Comparison of selected cytokine concentrations in serum, CSF, and microdialysate in patients with traumatic brain injury.

Cytokine	Mean recovered MD concentration from ECF (pg/ml)	RR (%)	Mean recovered ECF concentration, adjusted for RR	Mean CSF concentration (pg/ml)	Mean serum concentration (pg/ml)
IL-1β	10.4–20.8	~30%	34.6–69.3	9.9–89	0.5–7.4
IL-1ra	2,796	~30%	9,320	26,861	10–221
VEGF	200.1	~4%	5,003	26–43	37–773

Concentrations of interleukin-1 beta (IL-1β), interleukin-1 receptor antagonist (IL-1ra) and vascular endothelial growth factor (VEGF) in cerebrospinal fluid (CSF), serum and microdialysate (MD) from extracellular fluid (ECF). The relative recovery (RR, in %) is noted and adjusted for. Data from Ref. (56, 58).

are both the source and target of these mediators (13, 57, 58), further complicating the interpretation.

Using either a qualitative approach or an unbiased quantitative analysis (such as PCA), cytokine production appears as an intrinsic and ordered component among the multiple biochemical pathways contributing to delayed brain damage (57, 59). This fact would argue against the inflammatory response being a secondary form of injury *per se*, and rather a sequential cascade of molecular events [in the words of Julius Caesar: *alea iacta est* (the die is cast)], with a predetermined sequence following the initial injury.

Perfusate for Cytokine Recovery Using Microdialysis

MD is commonly used in the NCCU for metabolic monitoring of the injured brain, recovering small metabolites such as lactate, pyruvate, glucose and glycerol from the ECF (45). For these metabolites, the relative recovery is circa 70% from catheters inserted into the brain, using a flow rate of 0.3 μl/min (60). Importantly, the pore size of MD membranes used for metabolite analysis is significantly smaller (20 kDa) compared to the size used for larger proteins such as cytokines (100 kDa) (61, 62). By using 100 kDa cutoff catheters with the standard crystalloid solution, the relative recovery for cytokines has been shown to be typically 30%, but as low as 5% for cytokines that oligomerize (e.g., TNF) (56). In order to improve poor recovery rates, colloids such as human albumin solution (HAS) or dextran may be added to the perfusion fluid (63). The addition of colloids does not always improve the recovery of all cytokines; however, the relative recovery for TNF in 3.5% HAS has been shown to increase from 4.4 to 31.2%, a sevenfold increase (56). A 3% Dextran-500 solution shows equally encouraging results compared to crystalloid solutions (64), and has the capacity to improve extraction of even larger inflammatory mediators (65, 66) and has been empirically demonstrated to remain within a 100 kDa membrane catheter (67). In summary, the optimal recovery of cytokines within the MD samples is dependent upon perfusate supplemented with albumin or dextran, favoring an improved relative recovery and no net loss of fluid from the catheter. In our opinion, this is an essential adjunct for protein microdialysis in general and specifically in recovering inflammatory mediators.

Statistical Approaches to Cytokine Studies

While the neuroinflammatory cascade following TBI is complex and characterized by substantial collinearity and functional overlap among mediators, many studies have focused on single cytokines utilizing univariate comparative statistics without adequately assessing the interrelationships with other factors (68–70). A more appropriate statistical approach would be to incorporate several cytokines into multivariate analysis methods, such as PCA (71). By using PCA, it is possible to detect the greatest sources of variance in the dataset and thus highlight patterns in the cytokine response (59). A regression extension of PCA such as partial least square discriminant analysis (PLS-DA) can be used to identify variations in a particular domain such as time (58, 72). PLS-DA has the disadvantage of introducing bias, but can be used to test prespecified hypotheses.

Interpretations of Cytokine Studies in TBI

A systematic review has identified 32 studies describing the analysis of CSF cytokines in 1,363 severe TBI patients, of which 19 studies reported the analysis of cytokines *via* MD in 267 severe TBI patients (73). The majority of the MD studies have been published from a few centers of excellence in TBI research, known for their work in the field of cerebral MD. From these studies, it is evident that MD catheter positioning plays a crucial role in the amount of cytokines recovered, with peri-lesional or lesional locations displaying greatly different cytokine profiles compared to "healthy" non-lesioned tissue. There is also a stereotyped response to injury whereby the peaks of MD derived cytokines such as interleukin-1 beta (IL-1β), IL-6, and IL-8 occur within the first 48–72 h post-TBI, with IL-10 remaining elevated throughout the acute period up to 5 days of monitoring. Therapeutic modulation of the neuroinflammatory response to TBI has been demonstrated clinically in patients using subcutaneous administration of recombinant human IL-1 receptor antagonist, with the aim of attenuating the experimentally proven IL-1-mediated cellular damage in the injured brain (57, 58). Several studies have attempted to correlate CSF cytokine concentrations [IL-1β, interleukin-1 receptor antagonist (IL-1ra), IL-6, IL-8, IL-10 and TNF] with patient outcome, demonstrating a number of conflicting data [summarized in a review article (74)]. We would argue that this approach is flawed as it ascribes a unitary role for a given cytokine (as "good" or "bad") as well as ignoring the high degree of collinearity between all these mediators (75). It is important to recognize the delicate interplay between mediators such that the ultimate effect of a given cytokine is determined by the specific cellular, chemical, and temporal milieu in which the cytokine is acting. Finally, the clinical complication profile with brain-ECF and CSF-based cytokine sampling appears to be quite low, attesting to the safety of both procedures in critically ill TBI patients. All these findings are necessarily limited by the small number of studies identified in the accompanying systematic review.

Interpretations of Cytokine Studies in SAH

Similar to the findings identified in the review on TBI, the studies reporting on the analysis of cytokines *via* MD and CSF in aneurysmal SAH cytokine can be found in the parent systematic review, included in this Research Topic in Frontiers in Neurology (Zeiler et al., 2017, II submitted). Overall, there are 9 studies describing the analysis of cytokines *via* MD in 246 aneurysmal SAH patients. Another 20 studies reported the analysis of CSF cytokines in 630 patients. As with the TBI cytokine literature, the MD and CSF studies identified within the review originated from a small number of centers of excellence in NCCU and SAH based research. This body of literature is smaller than that identified within the TBI population. Thus, the conclusions that can be made within the SAH systematic review are somewhat limited. However, as with the TBI literature, some interesting trends should be highlighted. Again, MD catheter location plays a role in the cytokine levels obtained in SAH patients, with lesioned tissue (i.e., ICH, ischemic brain) and peri-lesional tissue displaying higher cytokine levels than the "healthy" non-lesioned tissue. Second, SAH patients displayed peaks in MD IL-1β, IL-6, and IL-8 within the first 12 h' post-hemorrhage, while (as in TBI) IL-10 appears to remain elevated within the acute period. Third, CSF-based cytokines in SAH appear to correlate with patient outcome, but as with the TBI data, our interpretation is that this type of solitary comparative analysis for each given cytokine may be misleading as there will be a potent collinearity between all these mediators. The association between cytokine profiles and the incidence of clinical vasospasm post-SAH has been documented in a previous systematic review and meta-analysis, with IL-6 and TNF linked to the development of vasospasm and delayed ischemic neurological deficit (DIND) (76). In addition, chronic hydrocephalus and shunt dependency may be associated with TGF-β levels within the CSF for SAH patients.

TISSUE SAMPLING TO ASSESS NEUROINFLAMMATION

A direct method to determine focal inflammatory activity is to biopsy samples adjacent to the injured tissue. Compared to indirect sampling of fluid, this provides a measure of tissue inflammation with a high degree of confidence; however, it remains a temporal snapshot that cannot readily reflect dynamic processes. Harish et al. found that surgically removed tissue demonstrates higher concentrations of several, predominantly pro-inflammatory, cytokines, and chemokines in contused brain vs pericontusional tissue (77). They also found a high degree of macrophage and microglia activity in contused regions, suggesting strong inflammatory activity. Similarly, Bellander et al. identified increased complement activation from pericontusional tissue (78), indicating a strong inflammatory response, especially in surrounding neurons, following TBI. Additionally, in postmortem tissue of TBI patients, it has been shown that pro-inflammatory cytokine mRNA and protein concentration are significantly elevated compared to cytokines with a more anti-inflammatory function (79). In the same study, regions with a profound cytokine production were associated with abundant inflammatory cell infiltration, astrogliosis, and axonal pathology. While in postmortem tissue, changes that occur at the time of death might influence results, this study directly demonstrates that cytokines are upregulated in the brain parenchyma as early as a few minutes from brain injury. In the future, it might be possible to directly assess the inflammatory profile of the brain by acquiring brain tissue samples.

IMAGING TECHNIQUES TO MEASURE THE NEUROINFLAMMATORY RESPONSE

Advances in neuroimaging have allowed aspects of the neuroinflammatory cascade to be assessed with a high degree of spatial resolution. Ideally, these techniques can be combined with other tools such as MD, which are focal but provide temporal resolution, to get a more complete overview of inflammatory processes.

Positron Emission Tomography (PET)

Positron emission tomography relies on radioligands, which bind to a specific receptor or mimic a biological molecule of interest, and can be used in *in vivo* studies of neuroinflammation (80). Translocator protein (TSPO) is a 18 kDa mitochondrial membrane protein involved in steroid biosynthesis and is by far the most studied target to quantify microglial activation following TBI (81, 82). The first-generation TSPO ligand, [^{11}C]-PK11195 is the most commonly employed; however, there are now several second-generation agents with improved signal to noise ratio and reduced non-specific binding. The second-generation agents all demonstrate variable binding between individuals relating to a specific genetic polymorphism resulting in different TSPO affinity for the radiolabeled ligands (83). PET studies have revealed that the binding of TSPO ligand PK-11195 remains elevated chronically, many years following TBI (81, 82), and that the degree of binding, and therefore chronic microglial activation, correlates with the degree of cognitive impairment (81). The second-generation TSPO ligand [^{11}C]DPA-713 has been used to demonstrate cellular neuroinflammation in retired American football players (84, 85).

Translocator protein ligands are limited by their overexpression in reactive astrocytes, complicating the interpretation of the resulting binding (86, 87). Moreover, expense and the ability to generate radioligands on-site in the acute phase make the logistics of scanning complex. Nevertheless, currently, TSPO PET remains the only method for human *in vivo* imaging of microglial activation.

Other putative PET ligands to assess neuroinflammation include cannabinoid-2-receptor (88), cyclo-oxygenase (COX) (89), and matrix metalloproteinases (90) but these have not been adequately studied in acute brain injury. Furthermore, it is also possible that (^{18}F)-FDG-PET may be applicable to imaging neuroinflammation, because of the apparent linkage between metabolic programing and inflammation (see below).

Magnetic Resonance Imaging (MRI)

The clinically used MRI sequences may be used to explore specific features of neuroinflammation (91), most commonly by assessing BBB integrity through the addition of the contrast agent

gadolinium (92). In preclinical experimental settings, there are more advanced MRI applications, employing microparticles iron oxide, endothelial vascular cell adhesion molecule-1 (VCAM-1) (93), and macrophage-specific epitopes (94). However, in clinical studies in humans, MRI has not been employed to directly assess neuroinflammation, rather indirectly provide a measure of BBB dysfunction and endothelial cell activation.

Magnetic Resonance Spectroscopy (MRS)

The prevalent form of *in vivo* spectroscopy is ^{1}H magnetic resonance spectroscopy (^{1}H-MRS, also called proton MRS). This is employed to assess relative levels of cerebral metabolites. MRS is also known as NMR spectroscopy, but usually the term MRS refers to *in vivo* measurements and NMR to *ex vivo* or *in vitro* measurements. The most abundant signal in the brain ^{1}H spectrum is usually *N*-acetylaspartate (NAA). The peptide *N*-acetylaspartylglutamate (NAAG) is a product of NAA and the two are interconvertible. NAAG is a small peak in the brain ^{1}H-MRS that is difficult to distinguish from NAA. Often both are considered together as "total NAA" that is interpreted as a marker for neuronal health, viability, and/or number of neurons, particularly their mitochondria. Reduction in NAA is regarded as indicating dysfunction (permanent or temporary) of neuronal tissue. Other ^{1}H signals include creatine (combined signal from creatine and phosphocreatine), glutamate and glutamine (often considered together as Glx as the two species' signals are incompletely resolved), gamma-aminobutyric acid, and lactate. ^{1}H MRS also allows detection of elevated levels of myo-inositol (osmolyte present in glial cells) and choline containing compounds. Commonly, MRS is used to measure metabolic activity in TBI (95), but these substances have also been shown to act as a surrogate of neuroinflammation (91, 96).

Another form of MRS less commonly used is ^{13}C-MRS, which necessitates administration (usually intravenously, or sometimes orally) of substrates such as glucose, acetate, lactate etc. that have been manufactured to be artificially enriched in ^{13}C (e.g., 99%), in contrast to the natural abundance of ^{13}C that is only 1.1% of all carbons. ^{13}C-MRS methodology is a powerful means of quantifying metabolic fluxes *in vivo*, e.g., the tricarboxylic acid cycle and glutamate–glutamine cycling (97). Also, in recent years, ^{13}C hyperpolarization has evolved, a technique that boosts the ^{13}C-MRS signal albeit for a very brief duration. Clinically, it utilizes hyperpolarized 1-^{13}C pyruvate, given intravenously, to measure glycolysis vs TCA cycle, by means of signals for the respective products 1-^{13}C lactate and ^{13}C HCO_3^- (98). To date, it has mostly been used to image tumors (99), although results have emerged recently on ^{13}C hyperpolarization in preclinical TBI (100, 101). ^{13}C-MRS modalities may be useful in future in studies of neuroinflammation. A recent development (preclinically) is the use of hyperpolarized [6-^{13}C] arginine to detect inflammatory cell function in cancer (102), and this could presumably be used in brain injury. A final MRS modality is ^{31}P-MRS. No artificial enrichment is needed as ^{31}P is virtually 100% of all naturally occurring phosphorus atoms. ^{31}P-MRS is not commonly used, despite being able to measure energy-related phosphorus species non-invasively *in vivo*, including phosphocreatine, ATP, and inorganic phosphate. Notably, in a ^{31}P-MRS study of TBI patients, Garnett et al. (103) suggested that the changes (vs healthy controls) might be due to reactive gliosis in the injured brain. The ^{31}P-MRS modality is being actively investigated in acute brain injury.

THE ADAPTIVE IMMUNE SYSTEM

In comparison to the innate immune system, the adaptive immune response has not been as extensively studied. It is believed that the transition between the innate and adaptive immune response following acute brain injury is moderated by migrating antigen-presenting dendritic cells (104).

Cellular Components of the Adaptive Immune Response

The cellular components of the adaptive immune system can be measured in blood by identifying specific subpopulations of peripheral lymphocytes using flow cytometry. In severe TBI, this technology has revealed that the number of T-cells, specifically $CD8^+$ cytotoxic cells, decrease significantly following TBI, while the number of B-cells remain constant (105).

Autoimmune Responses to Neural Antigens

The humoral adaptive immune system is also activated following TBI as a result of presentation of previously novel neural antigens to the peripheral immune system. Acute brain injury triggers the production of autoantibodies toward brain-specific proteins, including the brain-enriched proteins glial fibrillary acidic protein (106) and S100B (107). Moreover, Cox and coworkers showed that peripheral blood mononuclear cells isolated from patients with TBI have the capacity to proliferate *in vitro* when stimulated by myelin basic protein, the most abundant protein of the myelin sheath expressed in the brain by oligodendrocytes (108).

These adaptive responses are long lasting and are a candidate mechanism in the later stages of the pathophysiology of brain injury that may underlie chronic neurodegeneration (109); however, the mechanism by which empirically identified autoantibodies inflict neuronal injury is yet to be proven.

FAILURE OF CLINICAL TRIALS USING "ANTI-"INFLAMMATORY AGENTS

There have been numerous failures to translate promising preclinical agents targeting the underlying conditions in brain injury studies into efficacious phase III human studies (110). Several reasons for this have been suggested, including small group sizes, inadequate dosing, inappropriate delivery route, inadequate therapy duration, and timing of drug delivery in relation to the occurrence of injury [several of the suggested harmful pro-inflammatory cytokines are elevated predominantly the first hours after injury (13)], the complexity of BBB and adequate drug penetration. In fact, large phase III studies, including corticosteroids (CRASH trial) (111), progesterone (112, 113), and erythropoietin (114) failed to detect the presence of the administered drug in the brain, something that could have

been determined empirically using microdialysis (45). Moreover, due to poor penetration of pharmacological compounds across the BBB, lower concentrations of the drug may have reached the brain explaining the lack of expected neuroprotection (115). Before deciding to embark on large phase III ventures, which are costly and consume much time and resources, adequate phase II clinical studies with informative surrogate endpoints should be performed, including microdialysis, to ascertain the degree to which the drug can cross the BBB and exert changes on relevant biomarkers, including immune response-related molecules such as cytokines and chemokines.

CONCEPTUAL UNDERSTANDING OF MECHANISM OF ACTION

Within recent years, there have been several ongoing trials aimed at negating the neuroinflammatory response in brain injury (43). As we improve the monitoring of the inflammatory response, we can improve the accuracy of prediction of how new anti-inflammatory drugs impact on the innate inflammatory response, and when they should be delivered, in order to best modulate neuroinflammation following brain injury. However, improved monitoring techniques also lead to increased study complexity as more data become available. Helmy and coworkers treated 10 patients with an IL-1ra (Anakinra) and they demonstrated that the cytokine response in ECF in the treated group resembled that of the classical pro-inflammatory microglial activation (M1) (116), with increased IL-1β and low levels of IL-4 and IL-10. These findings were counterintutitive given the classification of IL1ra as an "anti-inflammatory cytokine" and therefore more in keeping with the M2 (anti-inflammatory, adaptive) response that would have led to a predominance of anti-inflammatory cytokines (57). Nevertheless, the more data we obtain from the complex cascades in the neuroinflammatory response, the greater the refinement in our understanding. There is an increasing recognition that microglia and macrophages have the capability to express both M1 and M2 specific phenotypes (117), and that the classification system does not accurately reflect the complexity of microglial function (118).

Over recent years, the idea of metabolic reprogramming of macrophages associated with inflammatory phenotype has gained ground. Very recently, a study by Ip et al., in the context of inflammatory bowel disease, has shown that IL-10 opposes the switch to the more glycolytic metabolic program induced by inflammatory stimuli in macrophages (119). Specifically, IL-10 inhibits lipopolysaccharide-induced glucose uptake and glycolysis and promotes oxidative phosphorylation. These findings may be relevant to harnessing metabolic reprogramming to treat inflammation in injured brain.

CHRONIC SEQUELAE OF NEUROINFLAMMATION

Following brain injury, a chronic inflammatory state has been shown to correlate to several clinical conditions. One of the most common symptoms decreasing quality of life in patients that suffer from TBI is post-traumatic depression (PTD), which is present in up to 44% of patients (120). A study reported that higher levels of the acute CSF endothelial markers (sVCAM, sICAM-1, and sFAS) as well as IL-7 and IL-8, were associated with PTD as 6 and 12 months post-TBI, respectively (121).

Chronic traumatic encephalopathy (CTE) is a neurodegenerative condition leading to impairments in mood, behavior, cognition, and motor functioning and predominantly affecting patients with mild, repetitive TBI, common in contact sports (122). CTE has been defined as a tauopathy where phosphorylated tau accumulates within cells in the CNS (123). Recently, a cadaver study highlighted the presence of activated microglia (CD68-positive cells) close to tau deposits (124).

Another common complication following TBI is epilepsy (125), occurring in up to 20% of patients. Similarly, following SAH, a prevalence of between 7 and 25% has been reported (126, 127). Diamond et al. (128) found that a high CSF/plasma IL-1β ratio correlated with post-traumatic epilepsy (PTE) and that presence of a specific single-nucleotide peptide of the IL-1β gene (rs1143634) correlates with a higher risk of PTE following brain injury. Claassen and coworkers found an association between the inflammatory response following SAH (TNF-receptor 1 and high-sensitivity C-reactive protein) and the presence of seizures (129). Several reviews covering this field have been published, and all do suggest a strong link between neuroinflammatory cascades, increased excitotoxicity, and epileptogenesis in the aftermath of brain injury (130, 131).

It may therefore be possible to target inflammatory mediators after TBI and SAH, to alleviate the burden of these associated long-term morbidities.

COLLINEARITY AND CONFOUNDING FACTORS BETWEEN INFLAMMATORY MEDIATORS

Collinearity is a statistical term that describes how two (or more) variables correlate with each other to a high degree. As most inflammatory mediators are present at a low concentration in the uninjured brain, and the subsequent increase in concentration is a response to injury, they are likely to correlate with injury severity. It is therefore not surprising that many of the mediators can correlate with outcome and this may be confounded by the severity of injury, rather than a specific facet of the biology of the mediator of interest.

This is further complicated by the fact that most studies focus on a small panel of mediators such as the "pro"-inflammatory mediators such as IL-1β, IL-6, TNF, and IFN-γ as well as "anti"-inflammatory cytokines IL-4 and IL-10. However, we should not discount the importance of other unmeasured mediators in contributing to the detrimental inflammatory responses after TBI and SAH. Cytokines are known to act in complex cascades with some early potent mediators that subsequently trigger the synthesis of regulatory cytokines which act to temper the initial response. Although in some cases cytokines have partially overlapping functions, each cytokine has a unique role, pattern

TABLE 2 | Limitations in current neuroinflammation literature.

Issues	Limitations	Suggested approaches
Biological compartments	Brain-ECF best for receptor and brain tissue biology, while CSF easier to collect and available in larger volume. Both feasible only short term. Blood is readily accessible for any injury severity, multiple sampling possible but less specific for brain pathology. Direct tissue sampling has the highest spatial resolution but difficult to acquire	Combining multiple samples, microdialysate has specific advantages for drug studies but should be combined with serum/CSF to reflect the global production of mediators
Monitoring time frame	Varying time-frames to insult, some neuroinflammatory cytokines have brief early temporal profiles and thus late monitoring may miss some biological signals	– Correct to time of injury – Beware false negatives – Late inflammatory monitoring (6–12 months) is associated with chronic sequelae
Collinearity and confounding	Several studies only measure a small number of mediators and are thus inferring causation incorrectly as other mediators may confound results	– Multivariate statistics are necessary – Need to measure multiple mediators simultaneously to avoid bias – Interventional studies required to infer causation
Regional vs global monitoring	Signal to noise ratio with dilution of mediators vs missing focal lesions. How representative is the data?	Combinatorial approaches, e.g., focal monitoring, global biomarkers, and neuroimaging
Microdialysis methodology	Protein microdialysis requires specific approaches to improve relative recovery	– Dextran or albumin should be used as carriers to increase relative recovery – Sensitive assays necessary as low concentrations are common – Multiplex technology allows simultaneous measurement of several cytokines
Clinical follow-up	Clinical outcome metrics such as Extended Glasgow Outcome Scale, SF36 may be insensitive and fail to capture subtle neurocognitive sequelae	Several modalities of outcome assessment necessary after as long as 12 months after ictus, including – Cognitive – Neuropsychological – Quality of life – Psychiatric – Functional outcomes
Neuroimaging	Difficulties in inferring what NMR, and TSPO PET binding represents at cellular level in relation to neuroinflammation	Combinatorial approaches necessary for future research, e.g., focal microdialysis monitoring plus neuroimaging
Systemic injury	Polytrauma might contribute to peripheral inflammatory response, which may modify or overlap with central neuroinflammatory response	– Accurate definition of patient population and injury assessment – Measurement of brain compartments vs extracranial components
Tissue outcomes	Difficult to access tissue samples unless associated with a surgical procedure	Tissue biopsies present a way to accurately describe the focal inflammatory response. Can complement other techniques
Autoimmune response	Empirical evidence that the adaptive immune system involved, but not clear if epiphenomenon or causative in inflicting neuronal injury	– Relating innate to adaptive immunity is developing field – Issue of cellular elements vs humoral
SAH neuroinflammation	Several pathological entities can overlap: early brain injury, vascular spasm, tissue ischemia each with its own neuroinflammatory signature	Careful characterization of the clinical state at the time of monitoring
Preclinical experiments	Molecular and cellular events that drive neuroinflammatory responses in the acute and chronic phases in traumatic brain injury requires animal or *in vitro* models for better bed-to-bench side translational research	– Systematic consideration of age, weight, species, sex to highlight variations in the neuroinflammatory response – Large animal models necessary to replicate human injury patterns (gyrencephalic brain, greater volumes for sampling etc.) – Improved outcome metrics that are adequate representations of human conditions – Better collaborations between clinicians and preclinical researchers to address the caveats in current research paradigms

Table illustrating current issues, limitations, and suggested approaches.
MD, microdialysis; CSF, cerebrospinal fluid; SF36, short form questionnaire 36; NMR, nuclear magnetic resonance; TSPO, translocator protein; PET, positron emission tomography; SAH, subarachnoid hemorrhage.

of expression, and cellular source. Moreover, cytokines and chemokines display effects that are both "damaging" and "reparative" that may occur simultaneously in the post-injury phase (57). This dual effect has been also acknowledged for microglia and macrophages as both play both a beneficial and detrimental role in the injured brain (9, 14).

COMBINATION THERAPY

Biological redundancy in complex interrelated systems means that there are multiple pathways to secondary neuronal injury (45). However, in most clinical trials, only a single therapy is used to target a single pathway limiting the potential efficacy. This is a

potential reason why there is still no approved drug that has shown any clinical efficacy in mitigating neuronal injury in TBI and SAH. However, polytherapy using preclinically proven neuroprotective drugs (i.e., progesterone and Vitamin D hormone, creatinine, and choline) has also failed to show any treatment benefit (132), presumably as there are fundamental pathological differences between animal models and human brain injury (133) and also because we need a better understanding of the underlying pathophysiology and how the different pharmacological agents interact.

Nonetheless, a review of agents negating the neuroinflammatory cascades in brain injury indicates that there are many promising pharmaceutical agents currently being developed (43). Furthermore, with the tools currently available, it is increasingly feasible to monitor the neuroinflammatory response to therapy, thus allow researchers to rationalize specific combinations of therapy.

CHALLENGES AHEAD

There are several challenges and limitations in the field today. The major issues surrounding these techniques discussed in this manuscript are summarized in **Table 2**.

To measure and assess inflammatory activity, several current studies investigate a single marker in a single biological compartment. As discussed above, to adequately understand the neuroinflammatory response requires combinations of methodologies looking at multiple down-stream cytokines (59, 121), but also region specific focal inflammatory activity using PET-MRI (81, 82) and T-cell mediated autoreactivity toward brain-specific proteins (108).

Moreover, more sophisticated biostatistical methods analogous to the "-omic"-literature are required. Multivariate methods incorporating the entire cytokine profile using tools such as principal component analyses will presumably be necessary until we reach a greater understanding of these processes (57, 59).

In summary, we suggest a multimodal approach for the measurement of cytokines in various fluids, parallel to neuroimaging techniques to visualize changes in cellular/microglia activation with repeated measurement of both humoral and cellular immune activation to distinguish acute from chronic responses. Only this holistic approach will provide a deeper understanding of the longitudinal inflammatory processes in hope to develop accurately targeted and efficacious anti-inflammatory therapies.

CONCLUSION

The neuroinflammatory cascade following TBI and SAH comprises a wide array of both humoral and cellular players. An ever-growing literature has defined changes in cytokine production and cell activation in both the acute and chronic phases following brain injury. However, we are still confronted with the difficulty in placing the individual components into a coherent picture. The extended time course of inflammation following these conditions provides multiple opportunities for therapeutic intervention. However, there is a limit to what we can learn from observational clinical studies as to the role of individual mediators/cells and ultimately understand how they affect patient functional outcome. For this reason, it is pivotal to maintain a close dialog between clinical and experimental research, in order to identify the distinct role of cytokines and immune cells, acting in the large scheme of the complex inflammatory responses.

AUTHOR CONTRIBUTIONS

ET, AH, TT, FZ, MM-K, DM, PH, and KC designed and planned the study. ET, AH, TT, FZ, MM-K, DM, PH, and KC drafted the manuscript, which all authors read and approved.

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The Role of Substance P in Secondary Pathophysiology after Traumatic Brain Injury

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It has recently been shown that substance P (SP) plays a major role in the secondary injury process following traumatic brain injury (TBI), particularly with respect to neuroinflammation, increased blood–brain barrier (BBB) permeability, and edema formation. Edema formation is associated with the development of increased intracranial pressure (ICP) that has been widely associated with increased mortality and morbidity after neurotrauma. However, a pharmacological intervention to specifically reduce ICP is yet to be developed, with current interventions limited to osmotic therapy rather than addressing the cause of increased ICP. Given that previous publications have shown that SP, NK1 receptor antagonists reduce edema after TBI, more recent studies have examined whether these compounds might also reduce ICP and improve brain oxygenation after TBI. We discuss the results of these studies, which demonstrate that NK1 antagonists reduce posttraumatic ICP to near normal levels within 4 h of drug administration, as well as restoring brain oxygenation to near normal levels in the same time frame. The improvements in these parameters occurred in association with an improvement in BBB integrity to serum proteins, suggesting that SP-mediated increases in vascular permeability significantly contribute to the development of increased ICP after acute brain injury. NK1 antagonists may therefore provide a novel, mechanistically targeted approach to the management of increased ICP.

Keywords: substance P, traumatic brain injury, edema, intracranial pressure, brain oxygenation, sheep model

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Traumatic brain injury (TBI) has been identified as one of the leading causes of death and disability in individuals less than 40 years of age in developed countries (1, 2). Despite the significance of this public health issue, there is currently no accepted therapy that can improve outcome (3), largely because the pathophysiological factors and their mechanistic interaction in the injury process have not been well characterized. In addition to the primary (mechanical) injury caused at the time of the traumatic event, secondary injury factors play a major role in resultant neuronal cell death that results in lifelong disability experienced by many survivors. While a number of secondary injury factors have been identified (4, 5), water accumulation, or brain edema, has been recognized as being closely associated with patient outcome (6). Indeed, up to half of all deaths following TBI having been attributed to brain edema (7, 8). A number of treatment strategies have been introduced to relieve edema-associated brain swelling (9); however, these target the end result of the edematous process [increased intracranial pressure (ICP)] rather than the cause. These strategies include administration of hyperosmotic agents and barbiturates, hypothermia, hyperventilation, cerebrospinal fluid (CSF) drainage, and decompressive craniotomy (10). However, significant improvements in patient

mortality and morbidity have not been observed with these interventions, largely because they do not attenuate the specific mechanisms associated with edema formation after TBI.

Increased ICP can locally compress tissue, reduce cerebral perfusion, reduce brain oxygenation with resultant hypoxia and ischemia, result in brain herniation and, in severe cases, cause death. For ICP to increase after TBI, the volume within the confines of the skull must increase either through vasodilation, hemorrhage, increased CSF production, reduced CSF reabsorption, or edema. If ICP is increased because of edema, it follows that the total fluid volume of the cranial vault must have increased, and the only source of this water can be the vasculature (11, 12). Water movement from the cerebral vasculature to the brain parenchyma is known as vasogenic edema and has been well described as a significant component of early edema formation after TBI (6). Moreover, vasogenic edema is known to be permissive for subsequent cytotoxic edema (13), which then initiates a feedback loop that further drives increases in vasogenic edema and consequently ICP (12). Vasogenic edema requires that there is increased blood–brain barrier (BBB) permeability to serum proteins. A net movement of water from the vascular compartment then follows extravasation of blood plasma proteins into the brain parenchyma, leading to a disruption of fluid homeostasis. Given the increase in the volume of the brain tissue under these circumstances, ICP rises and negatively influences patient outcomes (14). In addition, the loss of barrier integrity following acute injury to the brain allows peripheral immune cells to cross the barrier and further contribute to and exacerbate the inflammatory processes within the brain (15).

Increased BBB permeability after TBI with subsequent edema formation has been recently linked to substance P (SP) release (16, 17). Moreover, the increased BBB permeability and edema formation after acute brain injury, together with the associated increased ICP, was reduced by administration of a SP, NK1 receptor antagonist (17–19). Accordingly, the current review focuses on the role of SP in TBI, particularly in relation to edema formation and increased ICP.

SUBSTANCE P

Substance P is peptide of 11 amino acids and belongs to the large tachykinin peptide family containing over 40 tachykinins, including neurokinin A, neurokinin B, neuropeptide-γ (NPγ), and the recently identified hemokinin 1. Originally identified in the 1930s by von Euler and Gaddum for its potent smooth muscle and hypotensive properties (20), SP is now known as a neurotransmitter that is released from primary afferent neurons in both the peripheral and central nervous system, as well as from non-neuronal cells such as inflammatory and endothelial cells (21, 22). In the nervous system, SP is localized to nuclei such as the substantia nigra and the medial amygdaloid nucleus, as well as to capsaicin-sensitive sensory neurons (23) where it is released in response to stimulation of the transient receptor potential channels by mechanical stimulation, temperature, pH changes, and ligand binding (24). Following release, SP can bind to tachykinin NK receptors to exert direct postsynaptic actions as a neurotransmitter or modulate other non-neuronal targets (22). The NK receptors are 7-transmembrane domain, G-protein-coupled receptors, with three, known as the NK1, NK2, and NK3 receptor, having been identified to date (21). Each of the tachykinin neuropeptides is able to bind all three receptor types depending on receptor availability and concentration of the neuropeptide, indicating that there is a degree of cross-reactivity among the receptors (21). SP normally has the highest affinity for the NK1 receptor, which given the predominance of the NK1 receptor in the adult brain (25) makes SP the tachykinin of particular interest in the context of CNS injury.

Synthesis

Two genes exist that are relevant to tachykinin synthesis, namely the preprotachykinin (PPT) A gene and the PPTB gene. Four different forms of mRNA are expressed through alternative splicing from the PPTA gene (26), the α and δ forms exclusively encoding for the synthesis of SP, while the β and γ forms encode synthesis of SP, as well as NKA, neuropeptide K (NPK), and NPγ; NPK and NPγ are elongated forms of NKA. In the brain, αPPTA expression is predominant, while in the peripheral tissues, βPPTA and γPPTA mRNAs are abundant (27). The PPTB gene encodes neurokinin B (22).

Similar to all neuropeptides, SP is synthesized on ribosomes that are exclusively present in the cell body. The mRNA encoding the tachykinin is initially translated into a larger protein precursor from which SP is subsequently released by the actions of proteases called convertases. Cleavage points for the convertases are doublets of cationic residues (26). After release, the actions of tachykinins are terminated by diffusion away from the receptor site or degradation by extracellular peptidases, the slow nature of these processes accounting for their prolonged effects (28).

Metabolism

Several enzymes are associated with SP metabolism including neutral endopeptidase (NEP) (29), angiotensin-converting enzyme (ACE) (30), SP-degrading enzyme (31), post-proline endopeptidase (32), dipeptidyl aminopeptide IV (33), cathepsin-D (34), and cathepsin-E (35). The individual cellular localization of NEP and/or ACE suggests that these enzymes are most likely responsible for the *in vivo* cleavage of SP (36), albeit that all of these enzymes have been shown to cleave the tachykinin *in vitro*. Hydrolysis of SP by both ACE and NEP removes the carboxyl terminal required for binding to the tachykinin receptors (30). NEP hydrolyzes SP in the peripheral tissues, brain, and spinal cord (37–39) with ACE-degrading SP in the plasma, CSF, and the substantia nigra (40), as well as contributing to the degradation of peptide fragments released by NEP.

Localization

Immunohistochemistry has shown that SP is present in the diencephalon, telencephalon, rhinencephalon, hippocampus, basal ganglia, pons, amygdala, hypothalamus, septal areas, myelencephalon mesencephalon, metencephalon, and spinal cord (41). SP immunopositive nerve fibers are common in most autonomic ganglia (42–44) and are detected in trigeminal and dorsal root ganglia (45, 46) as well as in intrinsic neurons of the gut (47). It is thought to play a modulatory role in the autonomic ganglia, the

best characterized response being observed in guinea pig inferior mesenteric ganglion where SP mimics a slow depolarization that can be evoked by repetitive afferent nerve stimulation (48). Peripheral inflammation has been shown to increase SP immunoreactivity in the superficial layers of the spinal cord (49) and increase release of SP (50). Damage to neurons or their intense activation also induces neuropeptide gene expression leading to alterations in neuropeptide biosynthesis (51). Specifically, during noxious stimulation or neurogenic inflammation in the periphery, there is an upregulation (28) of PPT mRNA expression (52) and NK1 receptor mRNA (53).

NEUROGENIC INFLAMMATION

Sensory nerve fibers positive for both SP and calcitonin gene-related peptide (CGRP) are found surrounding most blood vessels throughout the body. In particular, cerebral arteries have a rich supply of sensory neurons, suggesting that they have a role as mediators of the inflammatory process following injury. The release of these neuropeptides, including SP, is neurally elicited and results in a painful local inflammatory response known as neurogenic inflammation, characterized by increased vascular permeability, protein extravasation, mast cell degranulation, and vasodilation (54). These changes in vascular permeability and in blood vessel diameter result in localized swelling of the tissue (54). Also occurring are tissue-specific responses to neuropeptide stimulation including constriction of the bronchioles in the airways and contraction and/or relaxation of the smooth muscle in the bladder. SP is also widely considered to be the most active mediator of neurogenic inflammation, even though it is well known that other neuropeptides such as CGRP are involved. Nonetheless, CGRP potentiates the effects of SP by enhancing the bioavailability of SP through competition with SP for metabolism by endopeptidases and by increasing the expression of the NK1 tachykinin receptor (55). Neurogenic inflammation in itself also leads to an increase in the PPTA and NK1 receptor mRNA transcript (28), which encodes SP and its primary receptor, respectively.

A role for classical inflammation in the pathophysiology of secondary injury following TBI is well known (15); however, brain neurogenic inflammation has remained relatively unexplored until recently. First characterized in peripheral tissue, neurogenic inflammation has now been well described in a number of studies following acute brain injury (3, 16, 56). Its occurrence in the CNS was first demonstrated when electrical or chemical stimulation of the Dura mater, or acute capsaicin administration (a TRPV1 agonist), produced a local neurogenic inflammatory response in the form of increased protein extravasation that was not observed in the brain parenchyma itself or in the Pia mater (57). Subsequently in stroke, activation of endothelial NK1 receptors on blood vessels was shown to contribute to cerebral edema (58). Administration of SP was then shown in rats to produce a profound increase in plasma protein extravasation in the Dura mater, which was blocked when an NK1 receptor antagonist was administered and exacerbated by administration of either NEP or ACE inhibitors (59). In studies of TBI, inhibition of posttraumatic neurogenic inflammation by prior depletion of sensory neuropeptides using chronic capsaicin pretreatment attenuated increased BBB permeability, and the development of edema and functional deficits (60, 61), with subsequent studies demonstrating that ACE inhibitors exacerbated histological damage and functional deficits after TBI (62). Further studies in stroke established that reversible ischemic stroke resulted in increased brain perivascular immunoreactivity to SP with associated edema formation (63), while decreased SP immunoreactivity in association with increased NK1 immunoreactivity in both rat and human spinal cord injury suggested a role for neurogenic inflammation in this form of CNS injury (64, 65). Finally, activation of the multimodal TRPV1 receptor that is linked to SP release initiates neurogenic inflammation and is associated with increased BBB permeability, an effect abolished by the TRPV1 antagonist capsazepine and by an NK1 antagonist (66). Collectively, these data provide strong support that neurogenic inflammation involving the release of SP can occur within the setting of acute CNS injury.

SP IN TBI

Our own studies have shown that SP release is a ubiquitous feature of TBI and is associated with marked increases in BBB permeability, edema formation, and the development of functional deficits (56). Specifically, an increase in cerebral perivascular SP is observed following TBI as early as 5 h after TBI and persisting for at least 24 h following trauma (17). In human postmortem TBI tissue, the increased SP immunoreactivity colocalized with APP in perivascular nerve fibers suggesting that injury to these perivascular neurons was associated with SP release (67). The authors also reported that increased SP was apparent in cortical neurons and astrocytes, similar to observations made in the rodent models (17). SP mRNA levels as determined by PCR analysis remained elevated until at least 3 days posttrauma (68), suggesting persistent synthesis and release over this time frame. Moreover, serum levels of SP were elevated after TBI, with significant increases observed in both experimental (17) and human TBI, the latter having been associated with increased severity and mortality in patients (69). As discussed earlier, when SP catabolism is inhibited by the administration of an ACE inhibitor, further increases in SP immunoreactivity is observed together with an exacerbation of injury and neurological dysfunction (62).

Such increases in SP levels following trauma have been associated with increased permeability of the BBB and the formation of cerebral edema (12). Specifically, increased perivascular SP immunoreactivity after TBI colocalized with increased extravasation of Evan's blue dye, a marker of increased BBB permeability (17). The authors proposed that where SP was bound to a vascular endothelial cell, BBB permeability to vascular protein was increased. This increased BBB permeability to proteins was associated with the development of cerebral vasogenic edema, together with the development of persistent motor and cognitive deficits (17).

Further evidence supporting a role for SP in neurogenic inflammation after TBI has been obtained using NK1 antagonists (3). For example, the NK1 tachykinin receptor antagonist *N*-acetyl-L-tryptophan (NAT) attenuated increased BBB permeability, cerebral edema, and functional deficits when administered

at 30 min after TBI (17). Attenuation of BBB permeability toward Evan's blue was dose dependent and used to determine the optimal dose. The therapeutic window of the antagonist was established as 12 h with rats administered with the compound at such delayed time points still demonstrating reductions in neuronal injury and an improvement in functional outcome (70). However, only a membrane permeable form of the drug was effective at these later time points, suggesting that the efficacy at delayed time points was dependent on central penetration of the compound. These studies also established that inactive enantiomers of the active ligands were ineffective irrespective of the time point, emphasizing that neuroprotective efficacy was dependent on actual binding to the NK1 receptor and the inhibition of its activity.

Most experimental studies are confined to male animals, largely to avoid the confounding effects of gender related hormones. However, the efficacy of the NK1 antagonists in TBI has also been demonstrated in female animals (71). Specifically, increased SP immunoreactivity was again apparent after diffuse TBI and the NK1 antagonist, NAT, reduced BBB permeability to albumin, reduced axonal injury, and significantly improved functional outcome. Moreover, it reduced edema formation at 24 h after TBI by more than 80%. In related studies involving reversible ischemic stroke, administration of the NK1 antagonist at 4 h after stroke onset resulted in reduced BBB permeability and edema formation at 24 h, plus improved functional outcome over 1 week (18). Indeed, treatment with the NK1 antagonist was more effective than neuropeptide depletion with capsaicin pretreatment (72) and importantly did not reduce the effectiveness of tissue plasminogen activator (tPA) treatment (73). When combined with tPA, the NK1 antagonist actually appeared to stabilize the BBB and extend the therapeutic window of the tPA treatment, which in clinical scenarios has been limited to 4 h.

ICP AND BRAIN OXYGENATION AFTER TBI

Various animal models have been developed to reproduce aspects of the pathophysiology observed clinically after TBI, with rat models being the most widely used in experimental neurotrauma because they are considered cost effective and have readily available outcome measures (74). Disappointingly, however, treatments that have proven to be neuroprotective in these rodent models have not successfully translated to the clinical environment, thus emphasizing the importance of validating promising therapeutic agents in arguably more clinically relevant large animal models before progressing to clinical trials. Accordingly, we have developed a large animal model of diffuse TBI using sheep that reproduces consistent changes in ICP and brain tissue oxygenation ($P_{bt}O_2$) that are more representative of the clinical situation (75, 76). The use of a large animal, ovine model of TBI delivers a number of advantages that are not present in the more commonly used rodent models of TBI. First, there is the presence of a gyrencephalic brain with large white matter domains as opposed to a lissencephalic brain. The presence of gyri alters the mechanical response of the brain to TBI, while the large white matter domains alter the edema response. Sheep also have a significant tentorium cerebelli thus separating the brain into supratentorial and infratentorial compartments, similar to that observed in humans (77). These differences in cerebral folding, gray matter/white matter distribution, and brain compartmentalization may therefore contribute to the inability of some groups to consistently produce posttraumatic ICP responses in rats in the absence of mass lesions or hypoxia (78). Moreover, unlike rodents, sheep have remarkably similar ICP and $P_{bt}O_2$ values to humans, with normal ICP in the sheep between 6 and 9 mm Hg and $P_{bt}O_2$ being above 40 mm Hg, as well as the similar responses in these important physiological variables after TBI (75). Finally, large animals are amenable to using the same neurosurgical techniques and surgical instrumentation as that used clinically, which is also an advantage.

We have previously shown that ICP in moderate/severely injured sheep increases from control values of approximately 7 mm Hg to above 20 mm Hg within 1 h of the injury [(75); **Figure 1A**] and remains at those elevated levels in the hours that follow. This increase in ICP is consistent with the presence of vasogenic edema formation previously reported in diffuse TBI, and peaking between 4 and 6 h after injury (79, 80). Indeed, in the sheep brain, there was significant albumin extravasation at these early time points after TBI, confirming the presence of a more permeable BBB (80). When the NK1 antagonist, NAT, was administered at 30 min postinjury, there was a significant and sustained decline in ICP (19). Focusing on the time course of those previously reported changes [(19); **Figure 1A**], the NK1 receptor antagonist significantly reduced ICP by 32% within 3.5 h of administration, whereas ICP continued to increase by a further 36% in vehicle-treated animals. By 4 h after injury, ICP in the NK1-treated animals was half of vehicle-treated animals ($p < 0.001$) and approaching normal values (19).

The reoxygenation of brain tissue after TBI is an important part of effective therapeutic intervention, with restoration of aerobic energy metabolism essential to enable damaged tissue to recover. In the ovine model of diffuse TBI, injury typically results in a significant fall in $P_{bt}O_2$ to less than 50% of normal values, a reduction that persisted over the next few hours [(75); **Figure 1B**]. Administration of an NK1 receptor antagonist increased $P_{bt}O_2$ to more than 80% of control values by 4 h after TBI (81), consistent with the close relationship that has been previously described between ICP and $P_{bt}O_2$ after TBI (75). This improved oxygenation of the brain following administration of the NK1 antagonist may, in part, account for the improved neuronal cell survival and associated improvement on functional outcome previously reported (3).

A number of other experimental compounds have been successfully used to reduce edema formation in experimental TBI studies, including more recently progesterone and magnesium (4, 82), both of which have been unsuccessful in clinical trials to date (83, 84). Notably, when tested in our sheep model of TBI, our preliminary results also showed that both compounds had little effect on ICP or $P_{bt}O_2$ after TBI (unpublished results). The hyperosmotic compound mannitol is used clinically in the management of increased ICP following TBI, although considerable variability has been observed in both the ICP (9, 85) and $P_{bt}O_2$ response (86) in these clinical studies. The mechanism of action of mannitol is to draw water out of brain tissue into the vasculature where

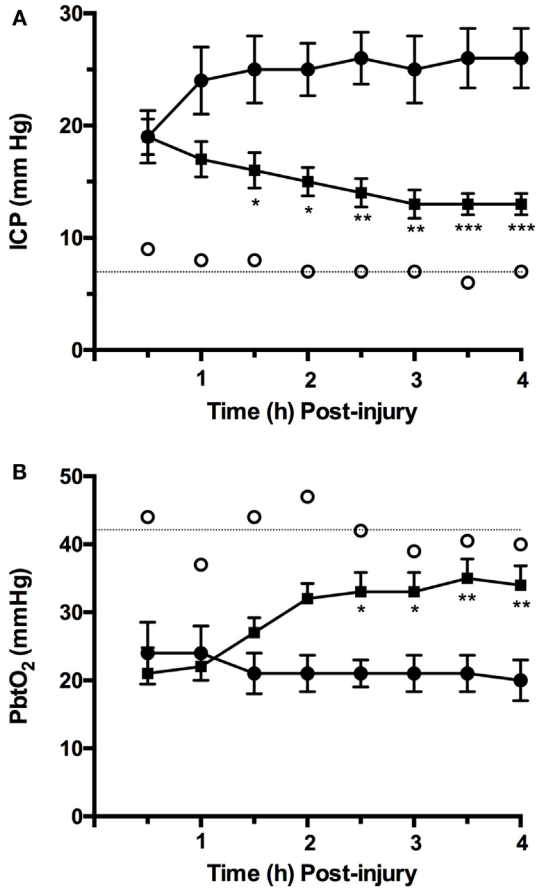

FIGURE 1 | Time course of changes in **(A)** intracranial pressure (ICP) and **(B)** $P_{bt}O_2$ following moderate to severe diffuse traumatic brain injury in sheep and treatment with an NK1 antagonist [adapted from Ref. (19, 75)]. Briefly, 2-year-old isoflurane anesthetized merino sheep were injured using the humane stunner and monitored for ICP and $P_{bt}O_2$. *N*-acetyl-L-tryptophan (NAT: 2.5 mg/kg i.v.) was administered at 30 min after injury. ○ = sham (uninjured) animals ($n = 9$); ● = vehicle (saline) treated animals ($n = 9$); ■ = NAT-treated animals ($n = 10$). *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$ versus vehicle-treated animals (two-way ANOVA followed by Bonferroni *post hoc* tests).

its presence has increased the osmotic pressure. This does not attenuate the potential mechanisms driving an increase in ICP, where the BBB after TBI is more permeable to proteins. There is also the potential that mannitol may actually cross into the brain parenchyma through the more permeable BBB, increasing the brain osmotic pressure and causing water influx with a rebound increase in ICP (87). By contrast, the mechanism of action of the NK1 receptor antagonist involves reducing BBB permeability and inhibiting the development of vasogenic edema (17), thus eliminating any possibility for a rebound increases in ICP.

MECHANISM OF ACTION

Vasogenic edema occurs when a BBB with increased permeability to vascular protein facilitates the influx of proteins such as albumin into the brain; vascular water subsequently follows down the osmotic gradient that has been created. A number of

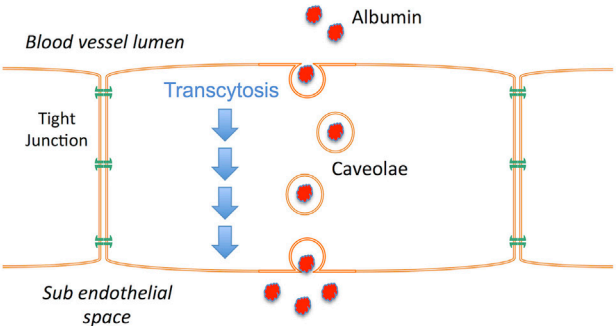

FIGURE 2 | Schematic illustrating the process of transcytosis where vascular proteins such as albumin are transported across the endothelial cells of the blood–brain barrier *via* caveolae. The creation of a protein osmotic gradient then drives water influx from the vasculature to the brain via aquaporin channels. NK1 receptors are present in caveolae.

events have been associated with both the early and late BBB disruption described after TBI, including classical inflammation, activation of matrix metalloproteinases, metabolic imbalances, and breakdown of tight junction proteins (88–90). However, the increased movement of proteins across the BBB does not require the physical breakdown of the barrier or the tight junctions, but rather can occur *via* caveolae in a process known as transcytosis (91). Indeed, in the hours following TBI, the tight junctions of the BBB have been shown to be intact (92, 93) as opposed to the caveolae that are upregulated (93). Increased caveolin-1 expression, a major constituent of caveolae, is thus thought to reflect an increase in albumin transcytosis after TBI (93), and account for the vasogenic edema that ensues (**Figure 2**). NK1 receptors have been shown to be located in caveolae (94–96), suggesting that their activation may play a role in regulating transcytosis. The fact that NK1 antagonists reduce BBB permeability after TBI, at a time when the tight junctions are intact and the BBB is more permeable to albumin, supports this suggestion. Thus, the release of perivascular SP after TBI activates NK1 receptors, including those localized to caveolae. This activates transcytotic albumin transport from the vasculature to the brain parenchyma, creating a protein osmotic gradient that drives water entry through aquaporin channels with subsequent edema formation. By inhibiting transcytosis, the NK1 antagonists attenuate the development of an osmotic gradient and negate the requirement for water movement from the vasculature to the brain. Without increased volume from vascular-derived water, there will not be an edema-associated increase in ICP, and any water that has accumulated in the brain will now be able to efflux *via* aquaporin channels (97).

CONCLUSION

NK1 antagonists reduce posttraumatic ICP to near normal levels within a few hours of drug administration. They also restore $P_{bt}O_2$ to normal levels in the same time frame, confirming an association between ICP and $P_{bt}O_2$ after TBI. The effects of NK1 antagonists on these parameters are more consistent and generally superior to mannitol, without the risk of rebound increases in ICP, and significantly better than the experimental

treatment strategies progesterone and magnesium, which are ineffective. We posit that SP-mediated increases in protein transcytosis increases vascular permeability, significantly contributing to the development of increased ICP after acute brain injury. Administration of NK1 antagonists reduces this protein transcytosis, eliminating the driver for vasogenic edema and thus reducing increased ICP. Accordingly, the NK1 antagonists warrant further investigation as a novel therapeutic approach to the management of increased ICP.

AUTHOR CONTRIBUTIONS

RV drafted the manuscript and prepared all of the figures. LG and ET contributed to **Figure 1** and drafted sections of the manuscript.

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Rethinking Neuroprotection in Severe Traumatic Brain Injury: Toward Bedside Neuroprotection

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Neuroprotection after traumatic brain injury (TBI) is an important goal pursued strenuously in the last 30 years. The acute cerebral injury triggers a cascade of biochemical events that may worsen the integrity, function, and connectivity of the brain cells and decrease the chance of functional recovery. A number of molecules acting against this deleterious cascade have been tested in the experimental setting, often with preliminary encouraging results. Unfortunately, clinical trials using those candidate neuroprotectants molecules have consistently produced disappointing results, highlighting the necessity of improving the research standards. Despite repeated failures in pharmacological neuroprotection, TBI treatment in neurointensive care units has achieved outcome improvement. It is likely that intensive treatment has contributed to this progress offering a different kind of neuroprotection, based on a careful prevention and limitations of intracranial and systemic threats. The natural course of acute brain damage, in fact, is often complicated by additional adverse events, like the development of intracranial hypertension, brain hypoxia, or hypoperfusion. All these events may lead to additional brain damage and worsen outcome. An approach designed for early identification and prompt correction of insults may, therefore, limit brain damage and improve results.

Keywords: traumatic brain injury, neuroprotection, animal models, intensive care unit, multimodal monitoring

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PANEL: KEY POINTS FOR IMPROVING PRECLINICAL RESEARCH

- Evaluation of drug effects in animal models considering different:
  - Types and severity of lesion
  - Species
  - Age, sex, comorbidities
- Assessment of early and late functional and histological outcomes
- Clinically relevant therapeutic window
- Well-elucidated mechanism of action and pharmacokinetics of the candidate compound
- Replication of the effect in different independent laboratories

INTRODUCTION

Progress in neurosurgery, neuroradiology, and critical care medicine in the last 50 years (1) contributed to the drops of 9% per decade in traumatic brain injury (TBI) mortality among hospitalized patients between 1970 and 1990, and it has been stable since (2). However, TBI remains a major cause of mortality and morbidity: approximately 57,000 deaths related to TBI occur in the European Union every year (3). Moreover, the increasing proportion of survivors includes many with neurological disabilities and poor quality of life (4); it was estimated that 7.7 million patients live with TBI-related disabilities in Europe (5). For all these reasons, neuroprotective strategies could provide immense benefits.

Even if there is no broadly accepted definition, neuroprotection in TBI can be considered as the body of interventions aimed at improving the patient's outcome, and preserving and restoring the integrity, function, and connectivity of the brain cells not irremediably damaged by the initial injury. While the primary injury at the moment of the impact (including hemorrhage, laceration, contusion, and primary axotomy) is not amenable to medical treatment, the complex cascade of molecular and cellular events (secondary injury) that follows the original damage can aggravate the initial harm. This cascade reduces the chances of functional recovery but could, at least theoretically, be counteracted (6, 7).

The first section of this paper discusses attempts to limit the progression of injury, focusing on preclinical research, and translational medicine. In the second section, we describe therapeutic interventions based on multimodal brain monitoring that could reduce the extent of additional insults to the injured brain.

NEUROPROTECTION IN PRECLINICAL RESEARCH AND TRANSLATIONAL MEDICINE

Traumatic brain injury is the result of an external force applied to the head (8) which, depending on the energy and site of the impact, can result in a number of different lesions commonly referred to as primary injury. Contusions, lacerations, epidural hematomas, subdural hematomas, subarachnoid hemorrhage, and axonal injury may be seen in TBI patients, based on the different mechanisms of the injury (direct impact, acceleration and deceleration forces, penetrating object, explosion blast waves), singly or in combination.

Beyond the brain tissue disruption at the moment of the impact, a broad spectrum of secondary events is triggered by the initial biomechanical injury. This include acute, subacute, and chronic events that all contribute to cell death and/or degeneration and are referred to as secondary brain injury (9). Briefly, alteration in ionic permeability and release of excitatory neurotransmitters (especially glutamate) propagate damage through energy failure and free radicals overload. Spreading depolarization is thought to be linked to this excessive release of glutamate. Cellular permeability is altered and increases calcium influx; this causes mitochondrial dysfunction, priming further energy defects, and apoptosis. Neurons ultimately may die through necrotic and apoptotic processes; autophagy is believed to play a role as well. Damaged axons may further fall prey to secondary axotomy and demyelination. Trauma directly affects the blood–brain barrier, with increased permeability causing protein-rich edema, and activation of a pro-inflammatory state. Inflammation, also promoted by resident microglia, has a dual action, spreading damage and, at the same time, promoting neurorestorative processes. This complex series of events starts immediately (seconds or minutes) after trauma but may last for weeks or months, especially inflammation (4). The contributions of each of these pathways to the secondary brain injury vary depending on the specific TBI lesion; for example, inflammation-mediated brain injury seems predominant in contusion while calcium-mediated injury predominates in diffuse axonal injury (10).

Several TBI models reproduce specific types of lesion in homogeneous groups of animals. Based on the distinct force applied, they can be used to investigate the components of the primary and secondary injury in time and space. Each model has specific advantages and limitations (9). None of them can be considered ideal, but together they lead to an understanding of mechanisms contributing to cell death or dysfunction after TBI. Consequently, experimental models have allowed the identification of therapeutic targets and the study of a wide spectrum of neuroprotective molecules including drugs aiming at specific targets (such as calcium-antagonists, NMDA-antagonists, free radical scavengers, bradykinin antagonists) and also drugs targeting multiple/pleiotropic mechanisms (such as anti-inflammatory steroids, erythropoietin, progesterone) (11).

Despite promising results in preclinical settings, these pharmacological neuroprotective compounds have proved disappointing in human studies. In the last three decades, more than 20 neuroprotective agents have been tested in clinical trials (11) without proof of significant outcome improvement. A recent overview of more than 10 "robust" studies (multicenter, including more than 100 patients, with an appropriate design and a low risk of bias) enrolling more than 15,000 patients confirms their failure to demonstrate any positive result (12).

This translational failure may have numerous reasons, both in the clinical and in the preclinical settings.

Subsequent reappraisals concerning the study design and conduction of clinical trials have been published (11, 13, 14). These analyses identified a number of possible critical factors that may have counteracted the neuroprotective potential of the compounds, for instance:

- the extent of side effects of the drugs and the occurrence of serious systemic complications
- the small sample size with inadequate statistical power
- the enrollment of patients too severe or too mild to detect any benefit
- the use of outcome scales insensitive to important consequence of brain injury
- the high heterogeneity of TBI population without the use of statistical tools for important covariate adjustment
- the inter-center variability in clinical care and clinical outcome.

To overcome this long list of limitations inherent to randomized clinical trials an alternative approach, devoted to Comparative

Effectiveness Research, is currently ongoing. An example is the Collaborative European NeuroTrauma Effectiveness Research in TBI (CENTER-TBI[1]), a large scale international project aiming at collection of demographic, clinical, imaging, genetic, and proteomic data from 5,400 TBI patients (15). The main objectives are to improve TBI characterization and classification and to identify the best clinical care, using comparative effectiveness research approach. CENTER-TBI and other similar projects running in Europe and North America, coordinated in the International Initiative for Traumatic Brain Injury Research (InTBIR[2]) are expected to provide high quality data, and rigorous statistical analysis, for improving care and outcome in TBI.

There is also the possibility that the preclinical findings were weaker than expected. First, there are limitations in the experimental models used, which exploit a single traumatic mechanism, such as direct impact or blast waves. However, no single mechanism can reproduce the wide pathophysiological and epidemiological heterogeneity of TBI—a very complex disorder. This means that a therapeutic effect detected in a homogeneous animal population exposed to a single type of injury may well not be generalizable to the human TBI population. Second, the quality of some experimental studies is variable (16, 17), and there is the risk of stressing positive results. It is recognized that blind assessment of the effect, animal randomization, and other indicators of quality are inversely related to the effect size in several published studies on brain injury (18). Third, the papers with negative results are less likely to be accepted for publication. This bias skews the literature and makes any thorough evaluation of treatment effects difficult or impossible (16–18).

In recent years, new approaches and research strategies have been proposed (19–21) to overcome these obstacles (see Panel). Operation Brain Trauma Therapy (21) moves in this direction. OBTT is a consortium of established preclinical TBI investigators supported by the US Army. It aims at identifying promising acute therapies for TBI, testing their efficacy across different animal models and laboratories through rigorous neurological examinations, motor and cognitive tests, brain and lesion volume measures, and biomarkers (22). OBTT recently reported that the neuroprotective effect of four (out of five) potential treatments, rigorously re-tested, was weaker than previously indicated in the literature (22). Those negative findings highlight the need for improving research standards in both preclinical and clinical research.

NEUROPROTECTION AT THE BEDSIDE

Repeated failures of pharmacological neuroprotective trials have blunted the enthusiasm for potential new wonder drugs. The number of industry-sponsored studies has markedly dropped, with eight new trials started in 1995–1999 but only one from 2005 to 2009 (13). Interestingly, failures occurred in the decades (1980–2010) during which the fundaments of neurocritical care were established and specialized care for severe brain injury emerged as a discipline.

The first textbook on "Neurological and Neurosurgical Intensive Care" was published by Alan Ropper and Sean Kennedy in 1983 (23). In 1995, the Society of Critical Care Medicine established a neuroscience section; in 2002, the NeuroCritical Care Society was founded. Growing interest in acute brain injury led to a pragmatic approach toward neuroprotection. While awaiting revolutionary pharmacological interventions, it became evident that additional, second insults after initial injury were frequent, and could be prevented and/or minimized in clinical practice. The hypothesis was that clear identification and correction of aggravating factors such as arterial hypotension could reduce the total burden imposed by TBI on the central nervous system and could consequently improve outcomes.

In this section, we describe some aspects of current ICU practice as part of a comprehensive strategy for minimizing insults to the injured brain and restoring brain homeostasis.

Early Phases after Brain Trauma: Hypoxia and Hypotension

Hypoxia (defined as arterial oxygen tension less than 60 mmHg or peripheral saturation of oxygen less than 90%) and hypotension (defined as systolic arterial blood pressure less than 90 mmHg) (24) in the early phases after TBI are frequent and dangerous insults (24–26). They are fundamental predictors of bad outcome (27, 28). Hypoxia may have multiple causes: direct traumatic pulmonary damage (contusion, pneumothorax, hemothorax), altered gas exchange (shunt, leakage because of increased capillary permeability), and lack of airway protection due to impaired consciousness. Hypotension is most frequently caused by massive hemorrhage or cardiac tamponade.

Hypoxia and hypotension may worsen outcomes through two fundamental mechanisms: they may be associated with severe extracranial lesions, such as irreversible shock, which on its own can worsen mortality. Then too, they may amplify the initial damage by impairing oxygen and glucose delivery to an already compromised brain (**Figure 1**).

Correction of hypoxia and hypotension with prompt airway management, support ventilation, and fluid resuscitation is mandated by international guidelines (29, 30). Airway protection with tracheal intubation, which has been debated in the past (31), is recommended in all patients suffering severe TBI (32). Hypotension must always be avoided and corrected with isotonic fluids and, when necessary, vasopressors. The long-standing debate on hypertonic fluid is still ongoing, with no evidence of superiority. On the other hand, albumin should be avoided. A recent re-analysis of the SAFE data (33) in the subgroup of TBI patients showed albumin infusion was directly related to higher intracranial pressure (ICP) and worse outcome (34). Blood components should be supplied, to optimize oxygen delivery with an adequate hemoglobin level and restore coagulation in hemorrhagic patients (35).

While no study has quantified the protective effect of careful avoidance of hypoxia and hypotension, the extent of additional damage they cause has been well documented in experimental conditions (36–38). A schematic revision of Traumatic Coma

[1]https://www.center-tbi.eu.
[2]https://intbir.nih.gov/.

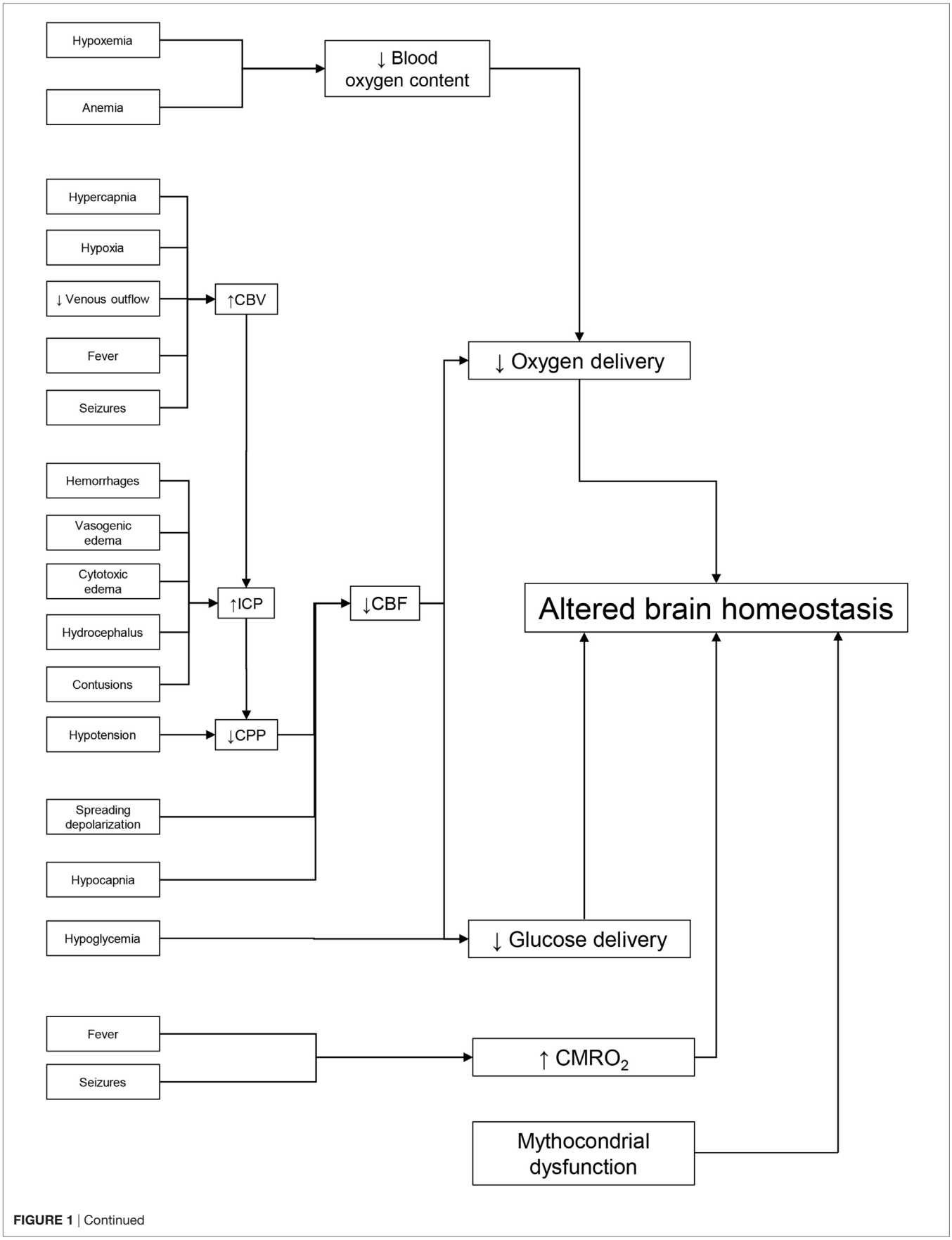

FIGURE 1 | Continued

FIGURE 1 | Continued
Dealing with potential brain insults at the bedside. Preservation of brain homeostasis requires careful detection of multiple threats, listed on the left side of the figure. Reduced delivery of metabolic substrate and/or increased cerebral metabolic rate of oxygen ($CMRO_2$) are the common pathophysiological mechanisms that may alter brain homeostasis. The key elements in oxygen delivery are blood oxygen content and cerebral blood flow (CBF). The first may be limited by hypoxia (secondary to respiratory failure) or by low hemoglobin. Continuous monitoring of CBF in the ICU is difficult but it can be estimated from cerebral perfusion pressure (CPP). Arterial hypotension and/or elevated intracranial pressure (ICP) [secondary to increased cerebral blood volume (CBV), hematomas, contusion, hydrocephalus, and edema] can reduce CBF. Cerebral vasoconstriction secondary to hypocapnia and spreading depolarization can also limit CBF. Glucose delivery is guaranteed by CBF and blood glucose levels. Factors limiting CBF and hypoglycemia (common during intensive insulin therapy) can reduce its supply. Seizures and fever are common causes of high $CMRO_2$ in the acute phase of traumatic brain injury. By leading to cerebral vasodilatation, they can raise CBV and ICP, lower CPP, and limit CBF and substrate delivery. Unfortunately, preserving the delivery of oxygen and glucose may not be enough to maintain cerebral homeostasis if their utilization is impaired by mitochondrial dysfunction.

Data Bank findings on 717 patients indicated mortality around 25%, in the absence of hypoxia and hypotension; this increased threefold for patients suffering hypoxia and hypotension (24). It seems likely, therefore, that preserving the injured brain from additional hypoxic–hypotensive insults could be beneficial.

ICP—Cerebral Perfusion Pressure (CPP)

A large amount of observational data (39–42) confirms the association between high ICP and unfavorable outcome, and particularly with increased mortality. High ICP may directly cause brainstem compression and distortion, which explains its relationship with mortality (43). It may also cause a critical reduction of CPP, leading to brain ischemia (**Figure 1**).

A recent South-American trial (44) on severe TBI, using a treatment strategy based on ICP monitoring compared with a clinical and CT-based strategy, failed to show better outcomes in the ICP group; nevertheless, the value of ICP monitoring still stands (45). Recent guidelines (46) have incorporated this trial, but still suggest ICP monitoring for reducing early mortality after TBI.

Cerebral perfusion pressure, calculated as mean arterial pressure minus ICP, is vital to perfuse the brain because it is the driving force for cerebral blood flow (CBF). The accepted threshold is commonly set at 60 mmHg (46, 47) but a higher threshold might be warranted in patients with impaired autoregulation due to chronic arterial hypertension (48).

The first strategy against dangerous ICP increases and CPP reductions is prompt recognition and removal of expanding intracranial hematomas (43). Reports based on few cases are extremely eloquent and prove that removal of subdural hematomas, while initially causing a destructive reduction of CPP and CBF (49) allowed restoration of cerebral perfusion (50). In this perspective, emergency surgery is an indisputable, effective neuroprotective strategy.

The treatment of increased ICP is based on a graded approach (43), with basic treatment (including sedation and supported ventilation) for all patients; more invasive therapy has to be reserved for more severe cases. Extreme therapies are only recommended for refractory intracranial hypertension, because of troublesome side effects. The concept of dosing therapy and applying more aggressive interventions only to selected patients is also evident from recent trials (51, 52). When highly invasive treatments, such as hypothermia or surgical decompression, were applied to patients with relatively low ICP, the outcome was worse in the treated group.

In clinical practice, careful ICP and CPP monitoring, coupled with tailored therapies, are fundamental neuroprotective tools: a first-line defense against brain stem compression and critical CBF reductions.

Advanced Intracranial Monitoring: $PbtO_2$ and Microdialysis

Inadequate substrate delivery (mainly oxygen and glucose) to the brain is an obvious cause of tissue hypoxia, metabolic disturbances, and potential metabolic crisis. Multimodal monitoring (53) offers the possibility of measuring (and possibly optimizing) several key metabolic parameters in limited volumes of the brain.

Partial brain tension of oxygen ($PbtO_2$) can be continuously measured with specific probes, and microdialysis can be used to sample the extracellular concentrations of glucose, lactate, and pyruvate at specified intervals, usually hourly (53, 54). Besides CPP, which may give indirect information on the global CBF driving pressure, these parameters may capture signs of hypoxia, hypoperfusion, and downstream metabolism disturbances. Reduction of $PbtO_2$ below a threshold of 20–25 mmHg is associated with worse outcome (55–57).

Microdialysis offers an insight on the metabolic profile of the brain; a normal lactate/pyruvate ratio (LPR) (usually lower than 25) indicates physiologic glucose utilization through the Krebs cycle. The LPR reflects the redox state of the brain (54). When measured together with the extracellular glucose concentration, and possibly with $PbtO_2$, different metabolic profiles can be identified. For instance, a low glucose concentration coupled with a high LPR and low $PbtO_2$ is consistent with ischemia; mitochondrial dysfunction is suspected when a normal glucose concentration and normal $PbtO_2$ are found simultaneously with a high LPR (58). Metabolic disturbances measured by microdialysis are linked with worse outcome after TBI (53, 59). Early or persistent oxidative metabolic dysfunction has been correlated with brain atrophy (60).

These advanced monitoring techniques may measure the adequacy of oxygen and substrate delivery to the brain and identify dangerous alterations. Additional information besides traditional surveillance (based only on ICP and CPP), as provided by $PbtO_2$, has given encouraging results (61). Therefore, advanced multimodal monitoring could improve insult detection at the bedside and contribute to better brain protection.

Brain Electrical Disturbances during the ICU Course after TBI

Traumatic brain lesions, particularly after penetrating injury, are a major risk factor for seizures (62). Guidelines recommend

early prophylaxis with phenytoin to prevent seizures in the first week after TBI (46). TBI patients are exposed to other electrical disturbances as well, such as non-convulsive status epilepticus (NCSE) and spreading depolarization. NCSE has been diagnosed with variable incidence in TBI series (63, 64), often regardless of the use of antiepileptic drugs.

Pathological waves of sustained depolarization that propagate through the cerebral gray matter are attracting increasing research interest. They are indicated as spreading depolarization (65) and are associated with microvascular and metabolic alterations. Their exact pathological role and the potential benefit of specific treatments are still under investigation.

Seizures and analogous electrical disturbances (65, 66) demand energy. Uncontrolled hyperactivity of neurons can induce or worsen a metabolic crisis in the injured brain. Therefore, prevention of seizures and appropriate monitoring of electric activity (in selected cases by continuous EEG) can help prevent, or disclose, noteworthy second insults (67), offering additional protection.

Fever

Hyperthermia is deleterious to the damaged brain (68, 69). It can exacerbate ischemic injury (by increasing the brain's metabolic demand) and may cause vasodilation of the cerebral vessels. This increases the brain–blood volume and may worsen ICP (70). Ample evidence indicates that fever is dangerous in TBI patients, worsening morbidity, and mortality (71).

While repeated trials have reported that hypothermia offers no benefit (51, 72), it is agreed that hyperthermia is definitely an insult after TBI. Careful temperature monitoring, and treatment of fever, may therefore reduce further brain damage in the acute phase.

CONCLUSION

The paradox of neuroprotection in TBI is that, despite a long list of potential neuroprotective agents active under experimental conditions, no compound has demonstrated protection in clinical trials. Analysis of clinical and preclinical trials has identified several gaps and improvements are certainly needed. However, even the most rigorous scrutiny of evidence and the highest research standard, as proposed by OBTT, do not guarantee success: a similar initiative in ischemic stroke (73) led to a negative clinical trial (74).

While awaiting an effective molecule limiting secondary brain injury after trauma, good-quality neurointensive care can provide modest but effective neuroprotection. By monitoring systemic and neurological parameters, intracranial and extracranial threats can be identified. In this way, effective targeted therapies become possible, and the burden of additional insults to the brain might be lightened.

AUTHOR CONTRIBUTIONS

TZ, MC, and NS designed the review, assembled a preliminary draft, and incorporated further contributions from each author into subsequent versions. All the authors revised it critically for important intellectual content and approved the final version.

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REVIEW
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Aspects on the Physiological and Biochemical Foundations of Neurocritical Care

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Neurocritical care (NCC) is a branch of intensive care medicine characterized by specific physiological and biochemical monitoring techniques necessary for identifying cerebral adverse events and for evaluating specific therapies. Information is primarily obtained from physiological variables related to intracranial pressure (ICP) and cerebral blood flow (CBF) and from physiological and biochemical variables related to cerebral energy metabolism. Non-surgical therapies developed for treating increased ICP are based on knowledge regarding transport of water across the intact and injured blood–brain barrier (BBB) and the regulation of CBF. Brain volume is strictly controlled as the BBB permeability to crystalloids is very low restricting net transport of water across the capillary wall. Cerebral pressure autoregulation prevents changes in intracranial blood volume and intracapillary hydrostatic pressure at variations in arterial blood pressure. Information regarding cerebral oxidative metabolism is obtained from measurements of brain tissue oxygen tension ($P_{bt}O_2$) and biochemical data obtained from intracerebral microdialysis. As interstitial lactate/pyruvate (LP) ratio instantaneously reflects shifts in intracellular cytoplasmatic redox state, it is an important indicator of compromised cerebral oxidative metabolism. The combined information obtained from $P_{bt}O_2$, LP ratio, and the pattern of biochemical variables reveals whether impaired oxidative metabolism is due to insufficient perfusion (ischemia) or mitochondrial dysfunction. Intracerebral microdialysis and $P_{bt}O_2$ give information from a very small volume of tissue. Accordingly, clinical interpretation of the data must be based on information of the probe location in relation to focal brain damage. Attempts to evaluate global cerebral energy state from microdialysis of intraventricular fluid and from the LP ratio of the draining venous blood have recently been presented. To be of clinical relevance, the information from all monitoring techniques should be presented bedside online. Accordingly, in the future, the chemical variables obtained from microdialysis will probably be analyzed by biochemical sensors.

Keywords: neurocritical care, intracranial pressure, cerebral blood flow, cerebral energy metabolism, microdialysis

INTRODUCTION

Critical—or intensive—care medicine is defined as a branch of medicine concerned with the diagnosis and treatment of life-threatening conditions requiring invasive monitoring and advanced pharmacological or technical organ support. After a serious polio epidemic with many deaths due to insufficient respiration, the first critical care unit in the world opened at Kommunehospitalet in Copenhagen in December 1953 (1). Since then, critical care medicine has gradually developed and holds today a central position within medicine (2). Neurocritical care (NCC) emerged as a separate branch in the 1980s. It was introduced to improve outcome in serious diseases of the nervous system, e.g., stroke, cerebral trauma, brain swelling, seizures, and central nervous infections. In addition to the components included in general critical care, NCC is especially focused on problems related to intracranial pressure (ICP), cerebral blood flow (CBF), and cerebral energy metabolism (3). During NCC, information regarding these physiological and biochemical variables may be obtained, displayed bedside and included in the clinical decision making. In this review, we present some of the most widely used monitoring techniques and discuss how they may be used and interpreted to evaluate and optimize therapy.

ICP MONITORING AND VOLUME REGULATION OF THE BRAIN

ICP Monitoring—Principles and Techniques

In the 1950s, Nils Lundberg developed the first clinical technique for continuous monitoring of ICP (4). He used a simple technique where a catheter was inserted into the cerebrospinal fluid (CSF) of the lateral ventricle through a frontal burr hole. The ventricular cannula was then *via* a strain gage pressure transducer connected to a potentiometer recorder, and the ICP was displayed bedside online. The technique has remained the "gold standard" for ICP monitoring (5) due to several advantages: as calibration is possible, the data obtained are accordingly always correct, and CSF may be drained to decrease ICP. The technique is not without risk but serious complications like hemorrhages and intraventricular infection can be kept at a low and acceptable level (5). However, it can be technically difficult to insert the ventricular catheter in patients with compressed or dislocated ventricles.

Techniques utilizing intra-parenchymal ICP recording circumvent some of these problems. Several technical solutions are available utilizing fiberoptic tips or micro strain gages. The fiberoptic sensor utilizes the path length change induced by pressure applied to a diaphragm. The miniaturized strain gage sensors have a foil responding with a change in resistance as stress is supplied and the strain gage element is connected to a half or complete Wheatstone bridge circuit. This technique is common for many pressure transducers used in medicine. A principle difference from the intraventricular technique is the inability to recalibrate the intra parenchymal transducers (5–8). In addition, these devises may measure a compartmentalized local pressure (9). Some of the transducers show a low zero drift and have very low complication rates (10, 11). A non-invasive technique of monitoring ICP to replace the invasive measures mentioned would seem attractive. To be of value during NCC, it must provide a continuous and accurate bedside measure of ICP. Presently, there is no such technique available (5, 12).

Volume Regulation of the Brain

Since the intracranial space constitutes an almost completely closed compartment, the dynamics of the ICP is determined by the physiological regulation of the contributing volumes: the CSF volume, the cerebral blood volume (CBV), and, most important, the volume of the brain tissue itself. In this section, we focus on the physiological regulation of brain tissue volume and in the subsequent section on the regulation of CBF we discus aspects on the regulation of CBV.

Like in other organs, the volume regulation of the brain is mainly determined by controlling the fluid exchange across the capillaries. Due to the blood brain barrier (BBB), the brain differs from other organs regarding these mechanisms. In addition to its other vital physiological functions, the BBB is the most important regulator of cerebral volume (13). The flux of water across a microvascular bed (J_v) is described by Eq. 1:

$$J_v = L_p \times A \times [\Delta P - \sum \sigma_s \times \Delta \prod_s] \quad (1)$$

L_p represents the specific permeability for water (hydraulic conductivity), A is the surface area available for fluid exchange, ΔP is the transcapillary hydrostatic pressure difference, $\Delta\Pi_s$ the transcapillary osmotic pressure difference, and σ_s the reflection coefficient of each solute (s) of the system. The product $L_p \times A$ is denoted hydraulic conductance and reflects the total capacity for fluid exchange. Transcapillary water exchange is thus determined by the following factors: the hydraulic conductance of the capillary wall ($L_p \times A$), the differences in hydrostatic pressure (ΔP) and osmotic pressure gradient ($\Delta\Pi_s$) across the capillary wall, and the endothelial component determining which solutes are reflected and will contribute to the osmotic pressure gradient (σ_s).

The effective osmotic pressure across a membrane that is partly permeable to the solutes is less than the theoretical osmotic pressure. The reflection coefficient (σ_s) accounts for this difference (13). The value of σ depends on the relative permeabilities of the membrane to water and to solute: if the membrane is impermeable to the solute but not to water σ equals 1, and if the permeability of the solute is identical to the diffusion coefficient in water σ equals 0. **Table 1** gives the reflections coefficients of some clinically important solutes for the BBB (14). Sodium and chloride, which are the two major solutes of biological fluids, have BBB reflection coefficients of 1.0. Water passing the BBB in any direction will thus be very dilute regarding crystalloids.

The magnitude of the hydraulic conductance ($L_p \times A$) describes the rate by which water is transferred across the BBB whenever there is driving force ($\Delta P - \sum\sigma \times \Delta\Pi$). The only way of inducing transcapillary filtration or absorption is accordingly to affect the balance between the hydrostatic and osmotic forces across the capillary membrane. Under physiological conditions, variations in cerebral perfusion pressure (CPP; CPP = MAP − ICP) and intracerebral capillary hydrostatic pressure are of limited

importance for brain volume. First, intracapillary pressure is physiologically tightly autoregulated (see Regulation of CBF) and variations in systemic blood pressure are generally not transmitted to cerebral capillaries. Second, as described above, transcapillary fluid exchange is effectively counteracted by the low permeability to crystalloids combined with their high osmotic pressure (≈5,700 mmHg) on both sides of the BBB (13). This contrasts to most other capillary regions where the osmotic pressure force is mainly derived from the difference between plasma and interstitial colloid osmotic pressure, which approximately balance the transcapillary hydrostatic pressure (≈20 mmHg). Water passing the BBB in any direction will accordingly not be accompanied by crystalloids. If, due to a difference in hydrostatic pressure, water passes the BBB an opposing osmotic gradient will immediately be created. Thus, the brain volume is under physiological conditions relatively independent of variations in intracapillary hydrostatic and colloid osmotic pressure (**Figure 1**). This volume control is, however, not unlimited. Although the reflection coefficient is about 1 for several solutes (**Table 1**) the half times for exchange of solutes like sodium and chloride indicate that a slow influx of solutes will in time dissipate the volume-controlling osmotic gradient. Accordingly, the long-term control of brain volume also depends on other mechanisms.

TABLE 1 | The reflection coefficients (see text) of various substances for the blood–brain barrier (BBB).

Solute	Reflection coefficient BBB
Urea	0.44–0.59
Glycerol	0.48
Mannitol	0.90
Sucrose	0.91–1.00
NaCl	1.00
Albumin	1.00

Data from Fenstermacher (13) and Staverman (14).

The lymphatic system plays an important role for volume regulation in most organs by draining interstitial fluid. Drainage of cerebral extracellular fluid has also been shown to occur *via* cranial nerves and spinal nerve roots (15, 16). This lymphatic drain has been suggested to be of importance for the immunoreactivity of the brain (16, 17) but is probably unimportant for cerebral volume regulation. The brain uses the CSF system as an alternative. The importance of this pathway for volume regulation is, however, disputed. Some studies have indicated that due to the tortuous nature of the extracellular space bulk fluid flow is negligible during physiological conditions and there are conflicting data regarding bulk flow between white matter and CSF in normal animals (18–22). In experimental brain edema, transport of extracellular fluid into the ventricular space has been described (23), but this mechanism for removing edema fluid does not solve the primary problem—the formation of edema fluid by leaky cerebral microvessels.

Summary: ICP Monitoring and Volume Regulation of the Brain

Continuous, accurate monitoring of ICP and mean arterial pressure (MAP) is of fundamental importance for NCC. All non-surgical treatments of increased ICP are based on knowledge regarding the physiological volume regulation of the brain.

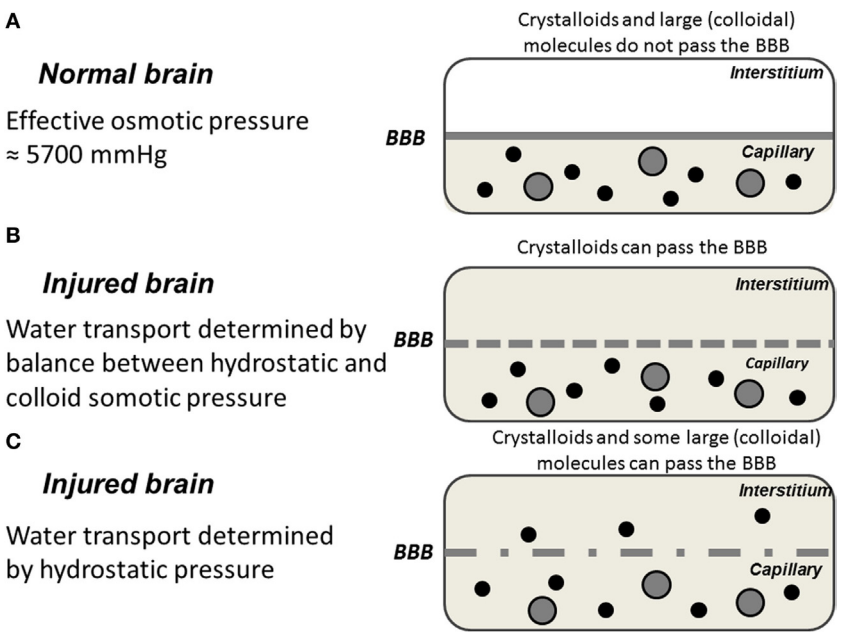


FIGURE 1 | Schematic illustration of water exchange across cerebral capillaries in three hypothetical situations: **(A)** the normal brain with intact blood–brain barrier (BBB); **(B)** the injured brain with a BBB permeable for crystalloids but not colloids; **(C)** the injured brain with a ruptured BBB permeable for crystalloids as well as colloids. Gray area represents crystalloids in the capillary; black circles represent large (colloidal) molecules; filled gray circles represent blood cells.

CBF AND CBF REGULATION

Principles and Techniques

A technique for quantitative, repeated measurements of CBF, which could be used under clinical conditions, was presented in the early 1960s. A radioactive tracer (^{85}Kr) was administered to the brain *via* the arterial blood supply, and its clearance was registered with an extracranial detector (24, 25). Later developments of the original technique have had a major impact on our knowledge regarding CBF and the regulation of CBF during physiological and patho-physiological conditions.

Mobile CT units have been developed to be used in NCC, and Xe-enhanced CT scanning gives useful information regarding regional CBF (26). However, as the CT scanner is not CE labeled, it is presently not available in Europe. Positron emission tomography and magnetic resonance tomography techniques are giving good information of CBF but do not allow for continuous measurements and can hardly be used in NCC. Accordingly, it may be concluded that there is presently no technique available for continuous, quantitative monitoring of global or regional CBF during NCC.

Many attempts have been made to measure CBF using ultrasound Doppler methods. However, the method gives blood flow velocities which should not be interpreted as blood flow. Laser Doppler technique, mainly used in research, is available but gives relative values and the measuring volume is small. The Bowman perfusion monitor (Hemedex®, Hemedex Inc., Cambridge, MA, USA) is a rather new device measuring focal CBF by a thermodilution technique and gives blood flow in ml/min/100 g (27, 28). The device has the drawback of being sensitive for temperature changes. The device can be used for brain temperature measurements.

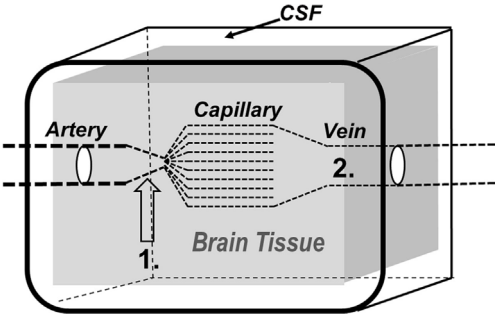


FIGURE 2 | Schematic illustration of the brain and its surroundings being enclosed in a rigid shell (CSF, cerebrospinal fluid). The vessels responsible for precapillary vascular resistance (1) as well as the intracerebral venous compartment (2) are indicated in the figure.

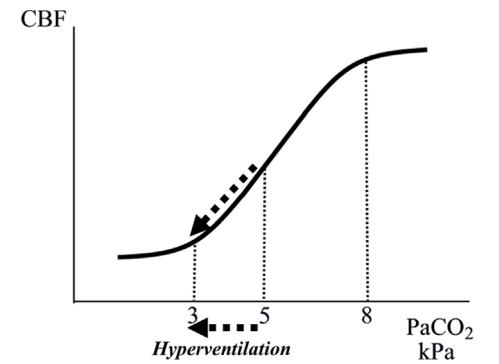


FIGURE 3 | Schematic illustration of the relation between arterial tension of CO_2 ($PaCO_2$) and cerebral blood flow (CBF). The interrupted arrows indicate the decrease in CBF during a decrease in $PaCO_2$ due to hyperventilation.

Regulation of CBF

The tight regulation of CBF secures a sufficient, continuous supply of glucose and oxygen to support the high metabolic demands of the brain (29). The control of CBF also affects two physiological parameters of importance for ICP: the intracapillary hydrostatic pressure and the intracerebral blood volume. The dynamics of ICP are closely related to three conventionally described physiological regulators of CBF: regulation by carbon dioxide (CO_2 regulation), metabolic regulation, and pressure autoregulation. All regulators of CBF primarily act by affecting the resistance at the precapillary vessel level (**Figure 2**).

CO_2 Regulation

Controlled normal ventilation ($PaCO_2$ 4.7–6.0 kPa) is currently the goal for severe traumatic brain injury (TBI) patients in the absence of cerebral herniation (30). Hyperventilation is frequently used during intensive care to achieve a rapid reduction of CBF leading to a reduction of CBV and ICP. This immediate effect (CO_2 regulation) is caused by a pH-dependent constriction of precapillary resistance vessels (**Figures 2** and **3**) (29, 31). However, because the perivascular increase in pH induced by hyperventilation is compensated metabolically within a few hours, the reduction of CBF and CBV is transient despite preserved hypocapnia. Accordingly, the decrease in ICP does not last (32). Pronounced hyperventilation may also carry a risk of inducing focal ischemia (33, 34), but the clinical importance of the potential risk remains controversial (35, 36). A pro/con debate on the whether $PaCO_2$ should be tightly controlled in all patients with acute brain injuries recently addressed this controversial issue (37).

Finally, as discussed later, as a result of impaired cerebrovascular CO_2 reactivity, hyperventilation often does not reduce CBV (and ICP) in patients with very severe brain injuries (38–40).

Metabolic Regulation

Figure 4 schematically illustrates the relation between cerebral metabolic rate and CBF. Data are based on experimental studies of epileptic seizures (41, 42), immobilization stress (43, 44), and administration of amphetamine (45) and barbiturate (46). Cerebral metabolic rate and CBF are generally reduced during coma (29). Hypoglycemic coma is an exception. In this condition, CBF is markedly increased (47).

A reduction of cerebral energy metabolism is usually accompanied by a lasting vasoconstriction and reduction of CBF and CBV (**Figure 4**). The effect may be induced pharmacologically—e.g., by administration of barbiturates (39)—on condition that

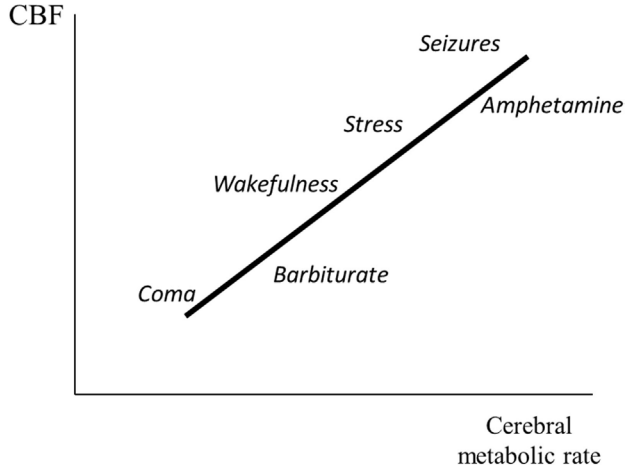


FIGURE 4 | Illustration of the relation between cerebral metabolic rate and cerebral blood flow (CBF). The figure gives a schematic summary of data obtained from experimental studies during induced epileptic seizures (41, 42) and immobilization stress (43, 44), and after administration of amphetamine (45) and phenobarbitone (46).

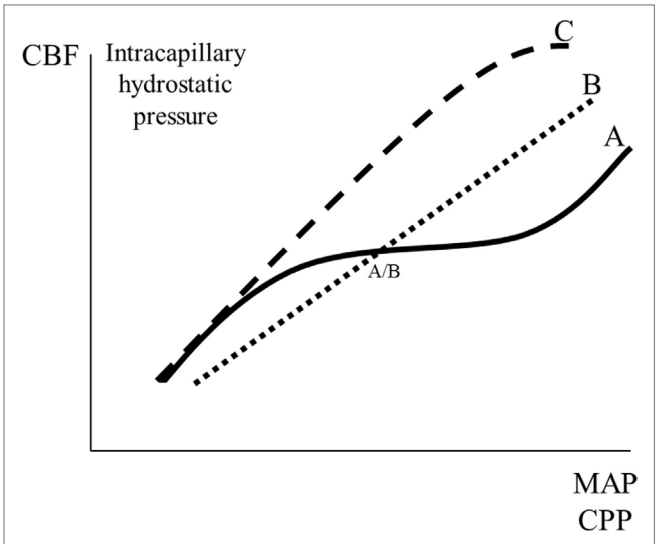


FIGURE 5 | Schematic illustration of cerebral pressure autoregulation of blood flow: the relation between mean arterial blood pressure (MAP)/cerebral perfusion pressure (CPP) and cerebral blood flow (CBF)/intracapillary hydrostatic pressure. Line A indicates intact autoregulation; line C indicates absence of autoregulation; line B indicates the description of absence of autoregulation often given in neurosurgical literature. The point A/B illustrates that in neurosurgical literature intact autoregulation is often interpreted as a mechanism preventing a decrease in CBF at a decrease in MAP/CPP.

cerebrovascular CO_2 reactivity is preserved (48, 49). The clinical usefulness of barbiturate coma is further reduced by the fact that prolonged high-dose barbiturate therapy is associated with pulmonary, cardiovascular, and other serious complications (50). Several other drugs that decrease cerebral energy metabolism also decrease CBF and CBV. Propofol has been used extensively for sedating patients when rapid awakening is desirable (51). However, propofol is associated with several complications and long-term propofol infusion has been related to severe adverse effects and mortality (52–55).

Pressure Autoregulation

Pressure autoregulation of CBF was first described by the Danish neurologist Mogens Fog (56, 57). As a result of the pressure autoregulation, CBF remains relatively constant, despite variations in perfusion pressure within certain limits (**Figure 5**) (56–58). In neurosurgical literature, the physiological importance of this mechanism is often interpreted as protecting the brain from a decline in CBF (**Figure 5**, line B) if CPP decreases below a certain limit (AB). In physiological literature, however, autoregulation is described as a mechanism that primarily serves to keep intracapillary hydrostatic pressure constant. In this literature, if autoregulation is impaired, then blood flow is described to increase passively according to line C in **Figure 5** (59, 60).

The vessels responsible for pressure autoregulation have been studied in experimental animals equipped with cranial windows for the direct observation of the cortical microcirculation (61). The large surface cerebral vessels (200–400 μm in diameter) were the most reactive to changes in blood pressure and were responsible for autoregulation between pressures of 120 and 160 mmHg. They also played the predominant role in minimizing changes in flow at pressures as low as 70–80 mmHg. The small surface and intracerebral arterioles did not change caliber when arterial pressure varied between 90 and 160 mmHg but, above 160 mmHg, they underwent forced dilation and contributed to the increase in blood flow observed.

Myogenic, metabolic, and neurogenic theories have been proposed to explain cerebral pressure autoregulation (62, 63). Additionally, endothelium-related factors have been suggested, and some studies have indicated a possible role for nitric oxide as a vasodilator during reduced CPP (64, 65). However, other studies have reported that nitric oxide may be a mediator of chemoregulation, but not autoregulation (66). Currently, it may be summarized that the mechanisms responsible for autoregulation are not completely understood and that the primary physiological function is to keep capillary hydrostatic pressure within narrow limits, despite variations in CPP. Additionally, vasodilatation may protect the brain from a fall in CBF during a moderate reduction in CPP.

For a quantitative evaluation of cerebrovascular resistance, it is necessary to measure blood flow and perfusion pressure. As there are no suitable techniques available for continuous monitoring of CBF during NCC and as autoregulation has been regarded as a clinically important phenomenon, various techniques for circumventing this problem have been presented (67). Of these, the so-called pressure reactivity index (PRx) has attracted most attention (68, 69).

Pressure reactivity index is based on continuous monitoring of the association between slow spontaneous waves in ICP and arterial blood pressure (ABP). It is calculated as a moving correlation coefficient between consecutive samples of values for ICP and ABP averaged for a defined period of time. For example, PRx may be calculated as the correlation coefficient between 30 consecutive average values over 10 s of MABP and ICP (altogether 5 min).

This 5-min window may be moved forward in increments of 12 s, generating five PRx values each minute and 1-min average for PRx may be calculated and presented (68). In studies, PRx may be averaged over long-time periods (2–6 h) for comparison with subsequent CBF measurements (69).

A problem with PRx is the fact that the index is not related to a well-defined physiological mechanism or process. Accordingly, the data obtained should not be regarded as a definite measure of vascular resistance or autoregulation. For example, during the time period studied, CBF may change for many reasons not related to variations in MAP.

In several studies, PRx has been shown to correlate to clinical outcome in severe brain trauma (68, 70). It has been suggested that PRx could be used for targeting the optimal CPP (71, 72) but currently the evidence is not sufficient to make recommendations for implementing this strategy (72). Furthermore, in patients with subarachnoid hemorrhage (SAH), the interpretation of PRx was ambiguous when compared to simultaneous measures of CBF, and it was concluded that PRx was not a reliable indicator of the status of autoregulation (69).

Summary: CBF and CBF Regulation

Presently, there is no technique available for quantitative, continuous monitoring of global or regional CBF during NCC. Three physiological mechanisms for regulation of CBF are of direct clinical importance: CO_2-regulation, metabolic regulation, and pressure autoregulation. These basic mechanisms constitute important parts of non-surgical therapy of increased ICP.

CEREBRAL OXYGENATION

As normal cerebral function is completely dependent on oxidative energy metabolism bedside techniques offering possibilities of monitoring cerebral oxygenation are of obvious interest. Three of these techniques will be presented and discussed: monitoring of jugular venous oxygen saturation ($SjvO_2$), brain tissue oxygen tension ($PbtO_2$), and near-infrared spectroscopy (NIRS).

Jugular Venous Saturation Monitoring

Monitoring of $SjvO_2$ has been used widely in various clinical conditions: TBI, SAH, during neurosurgical procedures and NCC (73). Placement of the $SjvO_2$ catheter is a relatively simple clinical routine procedure by a retrograde insertion of a jugular venous catheter. The tip of the catheter should be placed above the level of the C1/C2 disk to minimize the contamination from the facial vein (74). The choice of side is debated (75): the catheter can be placed on the side of the worst pathology, on the side where the internal jugular is most dominant, or bilaterally. Dominance may be determined by compression of each internal jugular vein separately and observation of the greater rise in ICP. Where there is no difference between the two sides, the right is commonly used as it is more likely to be the dominant side anatomically. $SjvO_2$ measurements can be obtained using intermittent blood samples or continuously with the use of fiberoptic catheters that use light wavelengths in the red/infrared spectrum to calculate saturation (74). Complications may occur during catheter insertion or due to prolonged duration *in situ*. Carotid artery puncture, hematoma formation, infection, thrombosis are possible complications but reported incidence is low (74).

Given stable arterial oxygen saturation and hemoglobin concentration $SjvO_2$ is interpreted as reflecting the balance between cerebral oxygen delivery (supply) and the cerebral metabolic rate of oxygen (demand). Accordingly, an increase in $SjvO_2$ may occur due to hyperemia (increased supply) or decreased $CMRO_2$ (decreased demand or inability to extract oxygen). A decrease in $SjvO_2$ may occur due to hypoperfusion (decreased supply) or increased metabolic activity (increased demand). Therefore, $SjvO_2$ may be considered as an indirect marker of CBF and cerebral metabolism (73, 76).

The normal range of $SjvO_2$ remains controversial. Generally, the lower range is considered to be 50–54% whereas 75% constitute the upper range (74–78). $SjvO_2$ desaturation <50% has been reported to be common following TBI associated with poorer outcome (74–78). Other clinical conditions that cause decreased $SjvO_2$ include decreased systemic oxygen supply, hypoperfusion (e.g., hypotension, vasospasm, intracranial hypertension), and increased cerebral metabolism or oxygen extraction (e.g., hyperthermia, seizures) (73, 74, 79).

For obvious reasons, $SjvO_2$ provides limited information in patients with focal cerebral ischemia. It has been estimated that an average of 170 ml of brain tissue was critically ischemic before $SjvO_2$ levels dropped below 50% (80). Accordingly, the correlation between $SjvO_2$ and $PbtO_2$ is often poor and high $SjvO_2$ has been reported in patients with low $PbtO_2$ and focal ischemia as well as in patients near to brain death (81, 82). Furthermore, it has been reported that $SjvO_2$ monitoring was useful for only 43% of the time that the catheter was *in situ*, and only half of the time that $PbtO_2$ monitoring was possible (83). Discrepancies found between $SjvO_2$ measurements between left and right hemisphere raise concerns also as to the choice of catheter location (75).

Brain Tissue Oxygen Tension Monitoring

Monitoring of $PbtO_2$ has been used extensively during NCC (TBI, SAH, cerebral infections) and during cerebral surgery (76, 84). The commercially available systems include Licox® (Integra Neurosciences, Plainsboro, NJ, USA) and Raumedic® (Raumedic AG, Helmbrechts, Germany). These probes come in different configurations, and the most advanced combines sensors for $PbtO_2$, temperature, and ICP within a single catheter (Neurovent-PTO), and the Licox® combines $PbtO_2$ and temperature sensors. Different principles for measuring the $PbtO_2$ are used in the two brands. The Raumedic® brand utilizes an optical method based on quenching of luminescence. Ruthenium is used as a luminophore and oxygen is the quencher. The Licox® is a polarographic electrochemical Clark-type cell electrode. This technique utilizes the electrochemical properties of noble metals to measure the surrounding oxygen partial pressure. The electrode consists of a membrane covering a layer of electrolyte and two metallic electrodes: oxygen diffuses through the membrane and is electrochemically reduced at the cathode. The greater the oxygen partial pressure, the more oxygen diffuses through the membrane. The change in voltage between the reference electrode and the measuring electrode is proportional to the amount of oxygen molecules reduced on the cathode. As the process is

temperature-dependent, a temperature probe is provided with the $PbtO_2$ probe to correct for variations in tissue temperature may differ in patients. The two commercially available systems have been compared during *in vitro* and *in vivo* conditions (85). Generally, the Raumedic® sensors measured higher $PbrO_2$ values (≈10%), but there was no significant difference regarding overall measurement or *in vitro* accuracy between the two probes.

$PbtO_2$ varies with changes in arterial oxygen tension (PaO_2) and CBF but exactly what $PbtO_2$ measures remains to be defined (86–88). In the extensive clinical study by Rosenthal et al. (88), it was concluded that the product of CBF and the arteriovenous difference in oxygen tension had the strongest relationship with $PbtO_2$. Several authors have, based mainly on clinical observations, suggested a threshold for $PbtO_2$ below which hypoxic/ischemic cerebral damage occurs (83, 89–93). However, in an experimental study, it was concluded that the threshold values for $PbtO_2$ under which energy metabolism fails was variable and most likely depending on the metabolic demands of the tissue (94). Accordingly, though $PbtO_2$ may accurately describe the tension of oxygen in the tissue—which is determined by the blood flow, the blood oxygen tension, and oxygen diffusion through the tissue—but does not disclose whether this oxygen tension is sufficient for maintaining adequate metabolism or not. To answer the latter question, it is necessary to measure cerebral energy metabolism (see Microdialysis).

A relatively large number of clinical studies have suggested a relation between measured $PbtO_2$ levels and mortality following TBI (83, 89–93, 95, 96). These studies have led to the hypothesis that $PbtO_2$ monitoring could be used for targeting and improving intensive care in severe TBI (97–100). However, two recent publications found that $PbtO_2$ guided therapy did not reduce mortality (101) and was associated with higher use of vasopressors and higher cumulative fluid balance leading to higher ICP and pulmonary edema (102).

Near-Infrared Spectroscopy

Near-infrared spectroscopy is based on the principle that near-infrared light penetrates tissues well. About 40 years ago, the differential absorption of near-infrared light by oxyhemoglobin and deoxyhemoglobin was noted, and the technique was suggested as method to measure circulation and oxygenation in the human brain non-invasively (103). As enzymes in the mitochondrial respiration chain also have differential characteristics of light absorption depending on their redox state (in particular cytochrome c oxidase), NIRS has also been suggested for estimation of cellular metabolism (104, 105). Today, the technique of NIRS has a number of different medical applications (106).

As NIRS is a continuous, non-invasive monitor, it has been used extensively for cerebral monitoring. When the distance between incoming radiation and the reflected radiation reaching the optical sensor at the surface of the head is 30 mm, the returned radiation will pass through a depth of approximately 20 mm of tissue. Accordingly, information derived from this reflection is limited to the cerebral cortex. As the scattering coefficient of this optical path is unknown, the oxygenated/deoxygenated hemoglobin level obtained is a relative rather than an absolute value. Normal range of cerebral regional oxygen saturation (rSO_2) has been reported to be between 60 and 75%, but individual baseline variation has been reported to be as high as 10% (107, 108). The lack of standardization among NIRS devices contributes to the difficulty of establishing definitive threshold levels. A drawback of the technique is also that there may be a portion of the oxygen content that derives from the scalp, bone, and meninges.

A number of factors may affect the NIRS signals adversely. NIRS appears to be most limited when there is preexisting brain injury. In TBI, the accuracy is often reduced by tissue swelling and the presence of extravascular blood collections in the subarachnoid, subdural, or intraparenchymal tissue compartments, the presence of subdural air after craniotomy, and a wet chamber between optical sensor and skin (109–111). Therefore, reports in TBI are conflicting. Some studies report that NIRS had a high failure rate and limited sensitivity in assessing rSO_2 in severe TBI (110) while others have found the opposite (112, 113). Although the indication for NIRS appears to be limited within NCC, the technique is frequently used in other clinical situations when cerebral oxygenation is of interest: extracorporeal circulation during open heart surgery, status post cardiac standstill. Accordingly, neuro-intensivists should be informed about the possibilities and limitations of the technique.

Comparison of $SjvO_2$, $PbtO_2$, and NIRS

The three techniques of $SjvO_2$, $PbtO_2$, and NIRS all permit bedside real-time, continuous data. The differences in information obtained is explained by the strengths and weaknesses of the three techniques and whether giving global ($SjvO_2$) or regional ($PbtO_2$, NIRS) information (83, 112, 113). Regional episodes of ischemia are accurately revealed by monitoring $PbtO_2$ (81, 83) but, as it is a local technique, the accuracy is dependent on the position of the catheter in relation to focal lesions. $SjvO_2$ and $PbtO_2$ values correlate closely in response to changes in global parameters such as brain oxygenation and CPP (83, 114). $SjvO_2$ gives information regarding global hemispheric oxygenation and may accordingly miss focal ischemia. These technical limitations should be considered when evaluating the estimation that a $SjvO_2$ threshold of 50% approximately corresponds to $PbtO_2$ of 8.5 mmHg (83). Correlation between NIRS and other measures of oxygenation is variable, and various results regarding such correlations have been reported (74, 78, 114). Of the three techniques, the impact of $PbtO_2$ for prediction of clinical outcome has been evaluated in most detail in TBI studies. In a prospective study of 53 patients with severe TBI, the authors found that the use of both ICP and brain tissue $PbtO_2$ monitors and therapy directed at brain tissue PO_2 was associated with reduced patient death following severe TBI (115). Furthermore, in a recently published study, the authors found that reaching a critical $PbtO_2$ threshold of ≤10 mmHg might be detrimental to cognitive outcomes following TBI (116).

Summary: Cerebral Oxygenation

All three brain oxygenation monitoring techniques exhibit specific advantages and limitations. $PbtO_2$ monitoring is probably currently the technique most frequently used in NCC. Monitoring cerebral oxygenation provide valuable physiological information that may help guide treatment. However, the interpretation

of these data rather than the monitoring itself will determine whether the patient will benefit from such technology.

TREATMENTS OF ELEVATED ICP

Physiological Aspects on Surgical Treatments

Because the intracranial space is surrounded by the rigid skull, an increase in volume of one of the intracranial components must be at the expense of others:

$$V_{\text{intracran}} = V_{\text{blood}} + V_{\text{brain}} + V_{\text{CSF}} + V_{\text{mass lesion}} \quad (2)$$

This basic principle behind this hypothesis was formulated more than two centuries ago when a Scottish physician, Alexander Monro (1733–1817) applied some of the principles of physics to the intracranial contents. The hypothesis was supported by experiments by a Scottish surgeon George Kellie (1720–1779). In its original form, the hypothesis had shortcomings that prompted modification by others. What finally came to be known as the Monro–Kellie doctrine, or hypothesis, is that the sum of volumes of brain, CSF, and intracranial blood is constant.

All surgical and non-surgical treatments of increased ICP aim at changing the volume of one or more of these components. The surgical treatments are directed toward three of these volumes: $V_{\text{mass lesion}}$, V_{CSF}, and $V_{\text{intracran}}$. Following the initial resuscitation, early evacuation of significant focal mass lesions is the single most important treatment in patients with severe brain trauma (117, 118). CSF drainage, though it may be questioned from a theoretical point of view, can be used to reduce the amount of CSF in patients with remaining elevated ICP in spite of other measures. It is included in the recommendations by the US Brain Trauma Foundation (119). Craniectomy to increase the $V_{\text{intracran}}$ is often advocated as a last resort for treatment of increased ICP (120, 121). All surgical treatments of increased ICP have one consequence in common: they all reduce P_{tissue}. In a pathophysiological situation with increased permeability of the BBB, a decrease in P_{tissue} leads to increased transcapillary water transport. The consequences are known to neurosurgeons: the gradual increase in ICP always observed after evacuation of a focal lesion; the collapse of the ventricles sometimes induced by ventricular drainage; the bulging of cerebral tissue through the craniectomy. Accordingly, the rapid change in volume obtained by surgery should always be combined with non-surgical treatments aimed at a slow and lasting reduction in brain water content (122–125).

Physiological Aspects on Non-Surgical Treatments

Transcapillary Absorption of Interstitial Water

From a physiological point of view, it is obvious that non-surgical treatments of increased ICP should primarily focus on transcapillary reabsorption of interstitial water. The only way of inducing transcapillary fluid absorption is by controlling the transcapillary osmotic and hydrostatic differences (see Volume Regulation of the Brain). Mannitol, urea, glycerol, and hypertonic saline are used during intensive care to decrease brain volume by osmotic withdrawal of water (cf. **Table 1**). The effects of mannitol infusion have been studied most widely. A rapid reduction of elevated ICP is usually obtained (126), but the effects of continuous or repeated infusions are not well documented. In experimental studies, multiple infusions of mannitol caused aggravation of cerebral edema resulting from interstitial accumulation (127). In addition to the osmotic effects, other possible benefits have been ascribed to mannitol, such as promoting CBF by decreasing blood viscosity (128) and scavenging of free radicals (129). However, no beneficial effect was obtained in a recent systematic review of mannitol therapy for increased ICP after acute ischemic stroke and cerebral parenchymal hemorrhage (130). A drawback of mannitol and urea is its shrinking effect of cells which promotes opening of the BBB.

As the intact BBB has a very low permeability for sodium chloride, intravenous infusion of hypertonic saline would be expected to decrease ICP. In experimental studies, a prompt and substantial decrease in cerebral water content has been documented after intravenous infusion of 7.5% saline solution (131), but the long-term effects are not well-documented. Several studies have shown beneficial effects of infusion of colloids in experimental brain trauma or stroke (132–136). A decrease in colloid oncotic pressure has been shown to aggravate brain edema after mild-to-moderate experimental brain trauma (137). In the "Lund Concept" presented later, preservation of the colloid osmotic pressure is obtained by albumin/plasma and red blood cell transfusions to normal albumin and hemoglobin values. In this protocol, albumin is the first choice because it is the main physiological colloidal substance of blood. Other colloids may be considered, but the use of human albumin is supported by the recent observation that albumin has a marked neuroprotective effect in experimental ischemic stroke (138).

The intracapillary hydrostatic pressure can be reduced by decreasing MAP, increasing precapillary vasoconstriction, or a combination of both. Both interventions reduce CBF, which may induce secondary injury—especially in vulnerable regions. This problem can be controlled at the bedside by monitoring regional cerebral energy metabolism. The physiological effects of reduced MAP are very different if the decrease results from reduced circulating blood volume or is caused by controlled antistress/antihypertensive treatment in a normovolemic patient (139). In the latter case, drugs (which affect peripheral circulation but do not cause intracranial vasodilatation) should be used. The combination of a β1-blocker (e.g., metoprolol) to reduce cardiac contractility and an α2-agonist (clonidine) to induce peripheral vasodilatation would be expected to be suitable, and CBF measurements in patients with severe head injury have documented that these drugs alone do not change cerebrovascular resistance (140).

BBB Permeability

In patients with peritumoral edema or interstitial edema surrounding a focal brain infection, corticosteroids are used to decrease BBB permeability. However, in patients with severe brain trauma, ischemic stroke, intracerebral hemorrhage, or aneurysmal SAH, large clinical trials have not shown any beneficial effects of steroid treatment (141–144), and the American Association of

Neurological Surgeons (AANS) concluded that the majority of available evidence indicates that steroids do not improve outcome or lower ICP in severe TBI (145). The reasons for the discrepancy between trauma and peritumoral edema are incompletely known, but it is reasonable to assume that the molecular mechanisms are different. The mechanisms leading to BBB breakdown and edema in trauma as wells in the peritumoral area have been studied extensively and were recently summarized in two comprehensive review articles (146, 147).

Experimental studies have indicated that low-dose continuous infusion of prostacyclin might improve cerebral microcirculation and reduce capillary permeability (148, 149). Some clinical studies have indicated a possible beneficial effect of prostacyclin in severe TBI (150–153). To summarize, there is presently no therapy with documented effect for reduction of increased BBB permeability in patients with TBI or stroke. However, several novel promising strategies targeting the BBB have been presented *in vitro* and *in vivo* animal studies, though their relevance and signaling pathways in humans remain to be proven (147).

Intracranial Blood Volume

Since long it has been known that ICP and intracranial blood volume is decreased during hyperventilation (154, 155). The effect is obtained by constriction of precapillary resistance vessels and always causes a reduction of CBF. **Figure 6A** shows the physiological effects of induced hyperventilation on global CBF, cerebral arterio-venous differences in oxygen content (AVDO), ICP, and cerebral vascular resistance (CVR) in patients with severe head injuries and preserved CO_2 reactivity (data from 48). In patients with impaired CO_2 reactivity (as defined by unchanged CBF), there was no change in CVR (**Figure 6B**) and the observed increase in AVDO and decrease in ICP were caused by regional

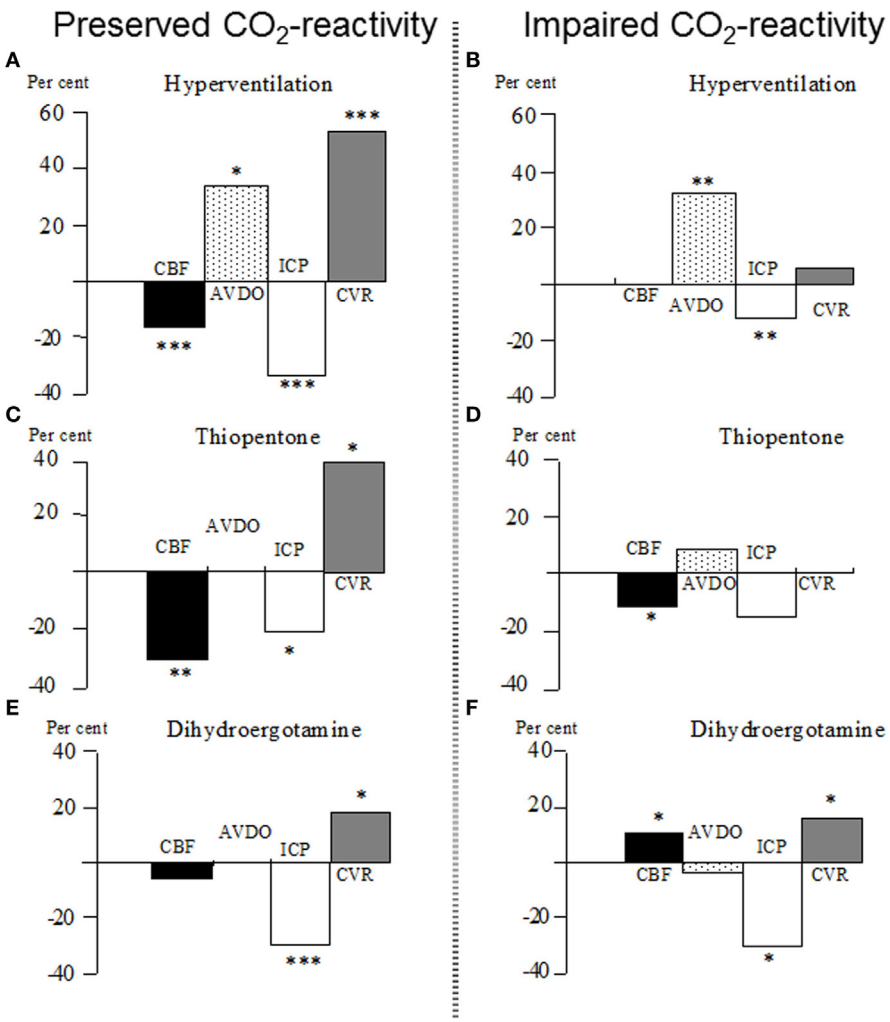


FIGURE 6 | **(A–F)** Percentage changes in global cerebral blood flow (CBF), cerebral arterio-venous difference in oxygen (AVDO), intracranial pressure (ICP), and cerebro-vascular resistance (CVR) in patients with severe brain trauma. After the initial test with increased controlled ventilation (hyperventilation), the patients were assigned either to the group "Preserved CO_2-reactivity" **(A)** or "Impaired CO_2-reactivity" **(B)**. After restoration of normoventilation, the patients were given either a bolus of thiopentone **(C,D)** (5–11 mg/kg intravenously) or a bolus of dihydroergotamine **(E,F)** (DHE; 4 mg/kg intravenously). $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$ for significance of difference from control value. Data from Ref. (48, 156).

differences in blood flow regulation not revealed by the global CBF technique (48).

The cerebral physiological effects of indomethacin are similar to those of hyperventilation, and the effects are long-lasting (157). In animal experiments, however, the reduction of ICP caused by indomethacin was always associated with signs of cerebral ischemia (158), and, consequently, the clinical usefulness of indomethacin therapy may thus be questioned. Barbiturate therapy also reduces ICP by inducing precapillary vasoconstriction. The vasoconstrictor effect obtained by barbiturate is caused by the reduction in cerebral energy metabolism. The decrease in CBF is paralleled by a reduction in energy metabolism (cf. **Figure 4**) and, accordingly, the vasoconstriction should not increase the risk of cerebral ischemia (48). **Figure 6C** illustrates that the decrease in CBF and increase in CVR are not associated with the increase in AVDO (data from 48). In patients with impaired CO_2 reactivity, the increase in CVR and the decrease in ICP were not obtained (**Figure 6D**). As these patients usually also have an impaired pressure autoregulation, the minor decrease in CBF was interpreted to result from a decrease in MAP (48).

A cerebral vasoconstrictor ideal for treatment of increased ICP should have its main effect within the venous compartment and less effect on precapillary resistance (cf. **Figure 2**). In the peripheral circulation, dihydroergotamine (DHE) is known to preferentially constrict venous capacitance vessels (159) and DHE has been shown to decrease ICP in patients with severe head injuries (160). This effect was obtained, although CBF remained unaffected or increased and no increase in AVDO was obtained (156, 161). **Figure 6E** summarizes the cerebral physiological effects for patients with preserved CO_2 reactivity (data from 156). Furthermore, as demonstrated in **Figure 6F**, DHE also decreased ICP in patients with impaired CO_2 reactivity (156), and *in vitro* studies have shown that DHE has a more pronounced constrictor effect on isolated human cortical veins than arteries (162). The latter observation explains why a cerebral vasoconstrictor can cause a fall in ICP, although CBF remains unaffected or increases. As DHE is a non-specific receptor stimulator, it may be associated with severe adverse effects and complications (163, 164). Sumatriptan is a more specific 5-HT stimulator. It has been shown to decrease ICP in animal experiments (162), but thus far, it has not been used in clinical studies.

In summary, induced hyperventilation is effective for a short-term decrease of ICP but is probably unsuitable for prolonged treatment. DHE decreases the ICP, and it does not reduce CBF or increase AVDO as its main effect is exerted on the venous compartment. However, DHE may cause severe side effects and complications. The ideal pharmacological vasoconstrictor remains to be defined but presently reduction of cerebral energy metabolism (usually obtained by infusion of a low-dose barbiturate) is the first choice.

The "Lund Concept" Therapy of Increased ICP

The "Lund concept" was originally developed to treat patients with severe brain trauma, impaired CO_2 reactivity, and a dangerous increase in ICP (122). In these patients, conventional therapies of increased ICP appeared to be ineffective (40, 48). The protocol has later been used in all patients with severe traumatic brain lesions as well as in patients with other cerebral disorders with increased ICP. For obvious reasons, the "Lund Concept" is not used in conditions with compromised arterial blood flow (e.g., arterial vasospasm after SAH).

In patients with head injury, the therapy begins after initial resuscitation and surgical evacuation of focal mass lesions. The patients are treated with controlled normoventilation (without muscle relaxation) under continuous monitoring of MAP and ICP. In 1995, the technique of intracerebral microdialysis and bedside biochemical analysis of cerebral energy metabolism was introduced to document that the therapy did not cause a dangerous decrease in CPP. The protocol followed in the "Lund Concept" is based on the physiological considerations mentioned earlier and can be summarized as follows.

Reduction of Stress Response and Cerebral Energy Metabolism

Stress response is initially reduced by liberal use of sedatives (benzodiazepines). A further reduction of the stress response and catecholamine release is obtained by a continuous infusion of low-dose thiopental (0.5–3 mg/kg/h) and fentanyl (2–5 μg/kg/h). The dose of thiopental is kept low to avoid cardiac inhibition, pulmonary complications, and other side effects (50). A protective effect of β1-blockade after head injury, through reduction of sympathetic nervous system effects on the heart and lungs have been suggested. Reports of the beneficial effect of β-blockade in head injury with regard to survival have been published (165–168).

Reduction of Capillary Hydrostatic Pressure

Mean arterial pressure is reduced to the physiological level for the age of the individual patient with a combination of the β1-antagonist metoprolol (0.2–0.3 mg/kg/day, intravenously or as a low continuous intravenous infusion) and the α2-agonist clonidine (0.4–0.8 μg/kg, 4–6 times/day, or a low dose of continuous, intravenous infusion) (125, 140). The antihypertensive treatment is initiated after evacuation of focal mass lesions when the patients are clearly normovolemic (as obtained by red blood cell and albumin/plasma transfusions to normal albumin and hemoglobin values and to a normal central venous pressure). A CPP of 60–70 mmHg is considered optimal; however, if necessary, a transient decrease in CPP (to 50 mmHg in adults and 40 mmHg for children) is accepted to control ICP (122).

Maintenance of Colloid Osmotic Pressure and Control of Fluid Balance

Red blood cell transfusions and albumin are administered to achieve normal values (Hemoglobin: 125–140 g/l, Serum-albumin: approximately 40 g/l) to ensure normovolemia and to optimize oxygen supply. The albumin/plasma/blood transfusions also serve the purpose of obtaining a normal colloid osmotic pressure, favoring transcapillary absorption. A balanced or moderately negative fluid balance is a part of the treatment protocol and is achieved by diuretics (furosemide) and albumin infusion. All patients are given a low-calorie enteral nutrition (max energy supply: 15–20 kcal/kg/day). These physiological principles

followed during NCC are supported by experimental studies (123, 125, 169–171).

Reduction of CBV

Intracranial blood volume may be reduced both on the arterial side with thiopental (48) and DHE (159–161) and on the venous side with DHE (156, 161). However, due to the serious complications related to DHE therapy (163, 164), this treatment is rarely used.

The "Vasoconstrictor Cascade" Therapy of Increased ICP

The "Lund Concept," advocating a reduction in CPP, was first presented in 1994 (125). In 1995, a contradictory protocol based on physiological considerations and advocating a pharmacologically induced increase in CPP as therapy for increase in ICP was published (172). The therapeutic principle was based on the so-called "Vasoconstrictor Cascade."

The theory was based on the hypothesis that in patients with a functioning pressure autoregulation an increase in MAP would, due to the induced vasoconstriction, cause a decrease in CBV and hence also a decrease in ICP (**Figure 7**). In patients with supposed impaired pressure autoregulation, it was assumed that the autoregulation curve was often shifted to a higher MAP level. By this mechanism, the authors postulated that marked increase in MAP from a vigorous use of vasopressors would result in a decrease in CBV and ICP: "Cerebral perfusion pressure management can serve as the primary goal in the treatment of traumatic intracranial hypertension with substantially improved mortality and morbidity following TBI. The minimum level of CPP in this instance is greater than 70 mm Hg and frequently higher, defined by individual circumstances that may occasionally require a level of 100 mm Hg or more, but average 85 mm Hg. Systemic hypertension and iatrogenic maintenance of CPP do not potentiate or worsen intracranial hypertension" (172).

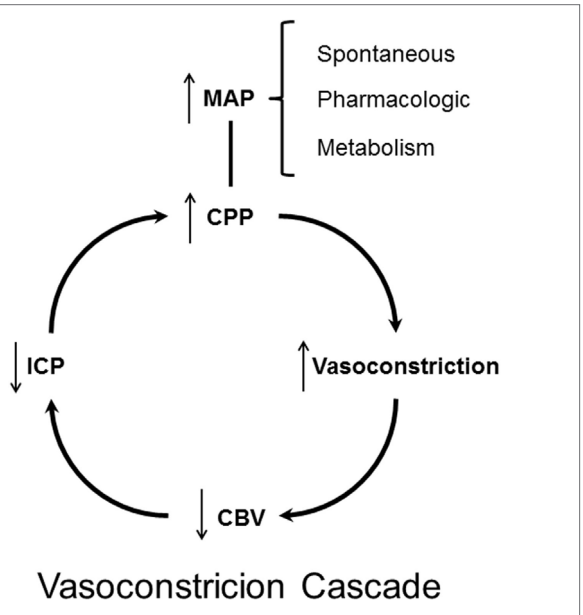


FIGURE 7 | Schematic illustration of the theoretical principles behind the "Vasoconstrictor Cascade" according to Rosner et al. (172).

Does the "Vasoconstrictor Cascade" comply with accepted physiological principles? By definition pressure autoregulation implies that CBF remains constant. CBF is controlled by the constriction/dilatation of the precapillary resistance vessels (**Figure 2**). As stated above, all surgical and non-surgical treatments of increased ICP act by changing the volume of one or more of these components. Assuming that the total intracranial volume is 1,500 ml (V_{blood} ≈75 ml) the distribution of the volumes of blood are approximately V_{venous} 50–55 ml, $V_{arterial}$ 10–20 ml, $V_{capillaries}$ 5–10 ml. Probably, the vessels responsible for autoregulation are small pial arteries with diameters between 200 and 400 μm (cf. above CBF, autoregulation). A decrease in diameter of these small vessels would accordingly cause a minor decrease in intracranial blood volume. However, by definition pressure autoregulation implies that CBF remains constant and, as discussed above, the decrease in CBV and ICP caused by constriction of precapillary resistance vessels occur at the expense of a reduction of CBF. Furthermore, according to the Poiseuille–Hagen equation, the flow through a vessel is proportionate to the radius to the fourth (R^4), while the volume is proportionate to R^2. Accordingly, it seems unlikely that preserved capacity for autoregulation and pharmacological increase in MAP could be useful for treatment of increased ICP. When tested in acutely head injured patients, it was documented that vasopressor therapy resulted in a marked increase in ICP in the group with severe brain trauma (126).

Summary: Treatments of Elevated ICP

All treatments of elevated ICP act by reducing one or more of the intracranial volumes (or increasing the total available volume through craniectomy). Non-surgical treatments act by decreasing brain and/or blood volume. The "Lund concept" is based on the physiological principles for regulation of these volumes. Many of these aspects are now included in protocols for treatment of increased CBF. However, it is still motivated to mention that the common belief that ICP may be decreased by an induced increase in MAP ("Vasoconstrictor cascade") is not supported by known physiological principles.

CEREBRAL ENERGY METABOLISM

The human brain constitutes only about 2% of the body weight but approximately 20% of the total body energy is consumed by the brain. To cover this very high energy demand, CBF constitutes 15% of cardiac output. The majority of the energy is used for active transport of various compounds against their concentration gradients, and it is estimated that less than 20% of the energy utilized by the brain is used for biosynthesis of cellular components. Due to the fact that glucose is the only substrate that is transported across the BBB at sufficient speed, glucose is under normal circumstances the sole substrate utilized by the brain. In the cytoplasm of the cells, glucose is converted to pyruvate (and lactate). The main part of the energy is obtained after pyruvate has entered into the citrate cycle in the mitochondria, ultimately being completely degraded to carbon dioxide and water. Global cerebral energy metabolism can be evaluated by measuring CBF

and arterial venous difference in oxygen content. These basic physiological and biochemical principles of cerebral energy metabolism have been known for decades and were described in a textbook in 1978 (29). However, as pathophysiological processes are often focal global, techniques for the evaluation of metabolism may give information of limited importance during NCC. Furthermore, it would be of clinical importance to monitor cerebral energy metabolism and indicators of cellular damage bedside. Intracerebral microdialysis is presently the only technique that offers this possibility.

Microdialysis

The microdialysis technique was developed more than 30 years ago for monitoring chemical events in the animal brain (173, 174) and has become an accepted scientific standard technique. Altogether, there have been well above 16,000 published studies utilizing microdialysis. In the late 1980s, the possibilities for monitoring the human brain were first explored (175–177), and microdialysis has since then been used for biochemical monitoring of most human tissues. In 1995, CMA Microdialysis (Stockholm, Sweden; present manufacturer M Dialysis, Stockholm, Sweden) introduced a sterile microdialysis catheter, a simple microdialysis pump and a bedside biochemical analyzer. The instrumentation was originally intended for subcutaneous and intramuscular use. With a slight modification of the microdialysis catheter, it has been used intracerebrally as an integrated part of routine multimodality monitoring since 1996.

The Microdialysis Technique

The original idea of microdialysis was to mimic the function of a blood capillary by inserting a thin dialysis tube in the tissue to analyze the chemical composition of the interstitial fluid. However, though the microdialysis catheter is thin (0.6 mm), it is much wider than a capillary and far bigger than the estimated intercellular distance (about 0.0001 mm). The wall of the catheter allows free diffusion of water and solutes between the surrounding interstitial fluid and the perfused solution (perfusate). The concentration gradients between the interstitial fluid and the perfusate constitute the driving force for diffusion. The molecular weight of the molecules being sampled is limited by the pore size of the dialysis membrane (cutoff). The perfusate flows along the dialysis membrane slowly and at a constant speed, and the sample (dialyzate) is collected in microvials and analyzed biochemically bedside by utilizing enzymatic, colorimetric techniques.

The achieved concentration of the analytes in the dialyzate depends on the degree of equilibration between the interstitial fluid and the perfusate. This relation is termed (relative) recovery and is defined as the dialyzate/interstitial concentration ratio expressed as percentage: Recovery = $Conc_{dialyzate}/Conc_{tissue}$.

Accordingly, the microdialysis technique does usually not give the absolute concentration of the studied biochemical variables. When clinical microdialysis is performed as a routine technique, this limitation is without significance. However, some of the factors determining the recovery are important to recognize.

The three most important factors affecting recovery *in vivo* are the area of the semi-permeable membrane (length of microdialysis membrane), the perfusion flow rate, and the diffusion in the surrounding interstitial fluid. The recovery increases in proportion to the length of the dialysis membrane area. The 70 Brain MD Catheter routinely used in the brain has a membrane length of 10 mm. The diameter of the probe is about 0.6 mm, and the standard cutoff of the dialysis membrane (during clinical routine) is 20 kDa. For special scientific purpose (monitoring of large molecules, e.g., cytokines), microdialysis catheters with 100 kDa cutoff are commercially available (178). The standard perfusion flow rate used during clinical routine is 0.3 µl/min, which allows sampling for bedside analysis every 30 min. Due to the slow perfusion rate and the large dialysis membranes, recovery is high: the *in vivo* recovery for the intracerebral 70 Brain MD Catheter is approximately 70% for the biochemical variables used routinely (179). If the perfusion rate is increased to permit more frequent sampling, recovery decreases to about 30% at 1 µl/min (179).

The diffusion rate in the surrounding interstitial space is of importance and varies with the molecular weight of the studied analytes and size and tortuousity of the interstitium. The recovery may thus vary between tissues and changes with the pathophysiological conditions. The problem is unimportant for clinical routine but is very relevant, for example when microdialysis is used for quantitative pharmacokinetic studies (180, 181). The importance of the diffusion limitation of the surrounding interstitial space also explains why it is useless to perform *in vitro* calibration to compensate for the recovery *in vivo*.

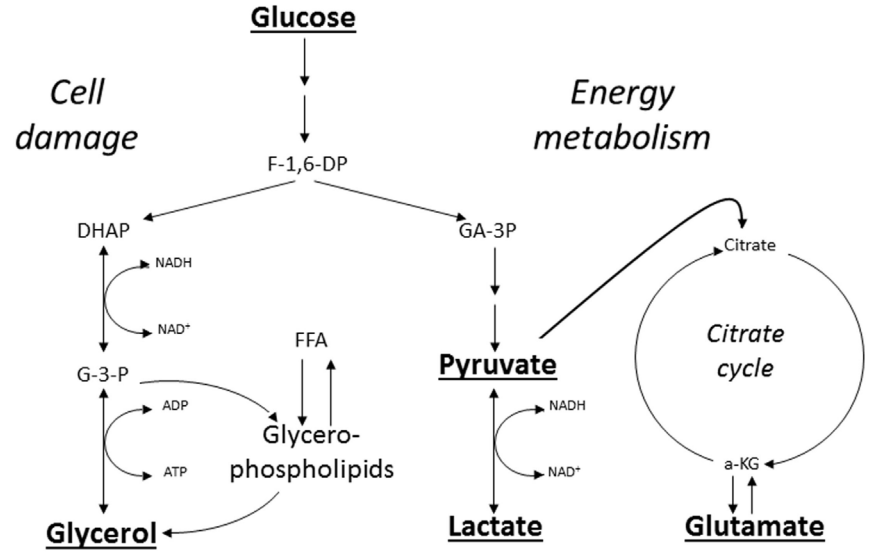
Biochemical Variables Monitored during Clinical Routine

The biochemical variables used for routine monitoring during clinical conditions were chosen to cover important aspects of cerebral energy metabolism and to give indications of degradation of cellular membranes. **Figure 8** shows these variables and their reference levels as obtained in normal human brain (182). Under normal conditions, glucose is the sole substrate for cerebral energy metabolism. In the cytosol, it is degraded to pyruvate (glycolysis) with a net yield of two ATP for each molecule of glucose. Due to the redox conditions, part of the pyruvate is converted to lactate. The lactate/pyruvate (LP) ratio reflects cytoplasmatic redoxstate, which can be expressed in terms of the lactate dehydrogenase equilibrium:

$$\frac{[\mathrm{NADH}]\,[\mathrm{H^+}]}{[\mathrm{NAD^+}]} = \frac{[\mathrm{Lactate}]}{[\mathrm{Pyruvate}]} \times K_{\mathrm{LDH}} \qquad (3)$$

Thus, the LP ratio gives information regarding the efficacy of cerebral oxidative energy metabolism. The major part of pyruvate enters the citric acid cycle in the mitochondria and is completely degraded to CO_2 and H_2O with a net yield of another 36 ATP. The chemical relation between the citric acid cycle and the excitatory transmitter glutamate is shown in **Figure 8**. However, the glutamate level obtained by microdialysis does not exclusively reflect liberation of the transmitter. As interstitial glutamate is normally rapidly taken up by the astrocytes against a concentration gradient (183), an increase in interstitial glutamate is often an indicator of jeopardized energy metabolism and release from leaky cells (184).

Since the brain does not contain any triglycerides (TG), a high level of intracerebral glycerol is considered to be a reliable



Normal human brain	Glucose	Lactate	Pyruvate	LP ratio	Glutamate	Glycerol
	1.7±0.9 mmol/L	2.9±0.9 mmol/L	166±47 µmol/L	23±4	16±16 µmol/L	80±40 µmol/L

FIGURE 8 | Schematic diagram of cerebral intermediary metabolism, with a focus on the glycolytic chain and its relation to glycerol and glycerophospholipids and to the citric acid cycle (Krebs cycle). F-1,6-DP: fructose-1,6-diposphate; DHAP, dihydroxyacetone-phosphate; GA-3P, glyceraldehyde-3-phosphate; G-3-P, glycerol-3-phosphate; FFA, free fatty acids; α-KG, α-ketoglutarate. Underlined metabolites are measured at the bedside with enzymatic techniques. References levels of the various metabolites for normal human brain obtained from Ref. (182).

indicator of degradation of the glyceropospholipids of cellular membranes and cell damage (185, 186). In other tissues, and in particular in fat tissue, glycerol is mainly obtained from degradation of TG. Lipolysis is under sympathetic control through catecholamine receptors on the adipocytes, which are stimulated by circulating catecholamines as well as by local noradrenergic nerve endings. The glycerol level in subcutaneous fat tissue may be used as an indicator of physical as well as mental stress (187).

During clinical routine, the biochemical variables may be analyzed bedside (ISCUSflex, MDialysis, Stockholm, Sweden). An analytical validation of the enzymatic techniques used for analysis of glucose, lactate, and pyruvate has recently been presented (188). For critical threshold, intra- and interassay coefficients of variation (CV) were, respectively, 3.1 and 4.5% for glucose, 3.5 and 4% for lactate, and 3.3 and 4.3% for pyruvate and inter-assay CV for LP ratio was 5.9%. The data prove that these routine analyses have the accuracy and precision required for clinical application in neurointensive care but the CV must be considered when these analytical techniques are used for scientific purpose (189).

Intracerebral Microdialysis in NCC

Though microdialysis has been used in most human tissues, the majority of clinical studies utilizing microdialysis have been performed in the brain. The biochemical variables routinely analyzed bedside were chosen to cover important aspects of cerebral energy metabolism (glucose, pyruvate, and lactate), to indicate excessive interstitial levels of excitatory transmitter substance (glutamate) and to give indications of degradation of cellular membranes (glycerol) (**Figure 8**). Most basic principles regarding cerebral energy have been known since decades (29) and similar patterns of changes have been described when utilizing intracerebral microdialysis. **Figure 9** shows changes in the intracerebral levels of glucose, lactate, and pyruvate after induction of cerebral ischemia, and in **Figure 10**, the simultaneous changes in LP ratio in the levels of glutamate and glycerol are shown (190). In this study, transient brain ischemia was induced in fetal lambs *in utero* by occlusion of the umbilical cord followed by resuscitation after cardiac standstill. The microdialysis technique was identical to that used during clinical conditions, but the perfusion rate was increased (1.0 µl/min) to allow frequent sampling. Induction of ischemia caused an almost instantaneous increase in the LP ratio shortly afterward followed by an increase of the glutamate level. Glucose, pyruvate, and glutamate rapidly recovered after resuscitation, but the levels of lactate and glycerol continued to be elevated and the LP ratio remained slightly above the pre-ischemic level.

These data are of importance for the interpretation of our clinical findings. The LP ratio, reflecting the redox state of the cytoplasm, will increase immediately when delivery of oxygen is insufficient and will rapidly return close to normal upon re-oxygenation. The lactate level rapidly increases during ischemia but remains elevated when circulation is restituted. Glycerol, the indicator of degradation of cellular membranes, increases relatively slowly during energy failure and remains elevated for some

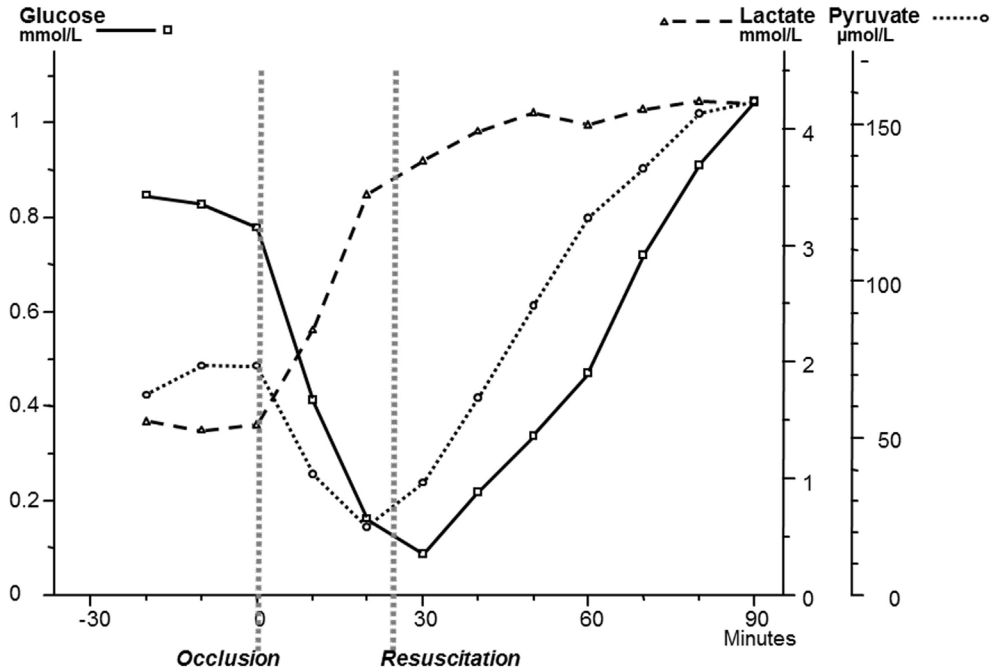


FIGURE 9 | Changes in intracerebral biochemistry during transient global cerebral ischemia. Levels of glucose, lactate, and pyruvate (mean ± SD). Data from Ref. (190).

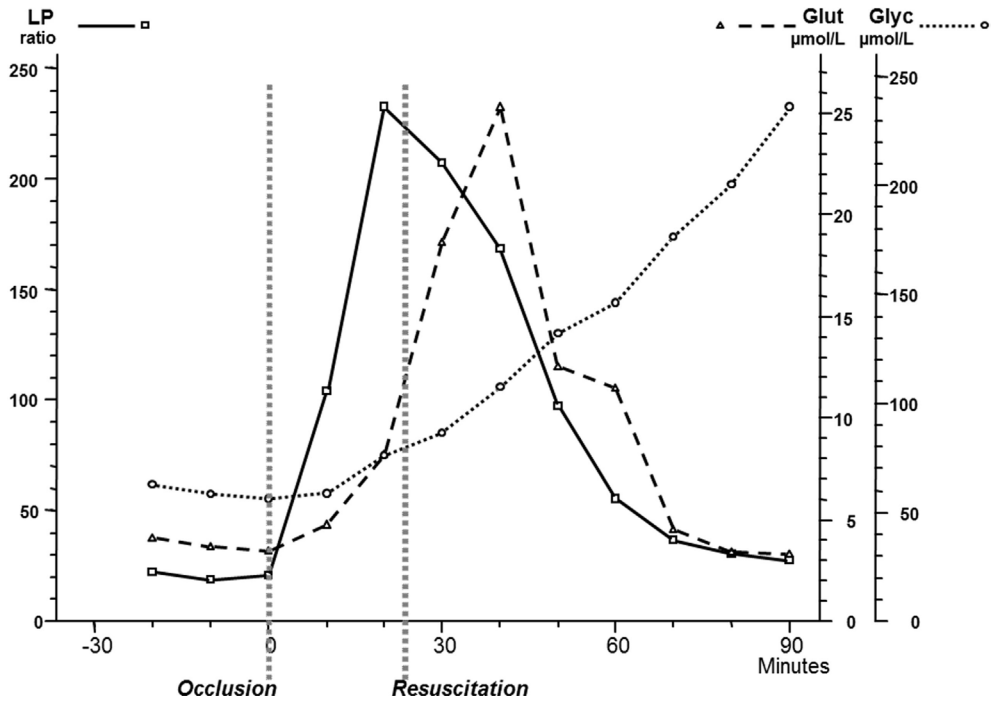


FIGURE 10 | Changes in intracerebral biochemistry during transient global cerebral ischemia. Levels of lactate/pyruvate (LP) ratio, glutamate, and glycerol (mean ± SD). Data from Ref. (190).

time when energy metabolism is normalized. The interstitial glucose level, finally, reflects the balance between delivery from the blood capillaries and the cellular uptake.

It is important to realize that the microdialysis technique gives biochemical information only concerning a small volume surrounding the catheter since regional differences in blood

flow and energy metabolism are considerable in most pathophysiological conditions. The fact that microdialysis is a regional technique may thus be regarded as an advantage provided that the positioning of the catheters can be visualized in relation to the focal injuries. Furthermore, one must be aware of the dynamics of the tissue condition which may considerable change with time (191). Thus, if the tissue targeted at catheter insertion was the "worst site," it may be less so after a while due to the evolution of tissue damage.

It has been possible to identify the metabolic pattern in various parts of the injured brain by inserting multiple intracerebral microdialysis catheters in patients with severe traumatic brain lesions. The studies have shown that "biochemical penumbra zones" surround focal brain lesions and that most adverse secondary events primarily affect these sensitive zones (192, 193). Thus, intracerebral microdialysis with bedside biochemical analysis may be used to detect and treat focal adverse events before they have caused cellular degradation or clinical deterioration that may be detected by conventional monitoring.

Intracerebral microdialysis has been used to determine the lower acceptable limit for CPP in the individual patient (194, 195) (**Figure 11**). As discussed previously, this level is of particular importance in patients with increased ICP partly due to brain edema. In these patients, a high CPP may increase the intracapillary hydrostatic pressure and cause a net transport of water into the brain tissue interstitium which will further increase ICP (see Volume Regulation of the Brain).

Bedside Diagnosis of Cerebral Ischemia and Mitochondrial Dysfunction

During NCC, microdialysis has primarily been focused on identifying episodes of secondary clinical ischemia. However, several studies have documented that during NCC prolonged disturbance of cerebral energy metabolism and increase of LP ratio were often not due to ischemia as cerebral oxygenation remained unaffected (196). Already in the 1970s, animal experiments had shown that transient cerebral ischemia often lead to a prolonged period of mitochondrial dysfunction (197, 198).

The biochemical pattern obtained during mitochondrial dysfunction has been described recently (199, 200). The results are shown schematically in **Figure 12** and compared with the corresponding metabolic pattern in cerebral ischemia. In cerebral ischemia, the interruption of blood flow and decrease in $P_{bt}O_2$ causes a very rapid increase in LP ratio (**Figures 9** and **12A**). As the cerebral delivery of substrate for energy metabolism (glucose) is also interrupted, pyruvate decreases to a very low level and, as a result, the LP ratio increases to extremely high levels. In mitochondrial dysfunction, $P_{bt}O_2$ is unchanged but, due to impaired mitochondrial function, oxidative metabolism is insufficient to meet the energy demands. The increase in glycolytic rate causes a massive production of lactate and increase in the LP ratio although tissue pyruvate remains at a normal level or increases slightly (**Figure 12B**). Under clinical conditions, an increase in LP ratio may be caused by a variety of mechanisms (201). Drugs that are effective in mitochondrial dysfunction are

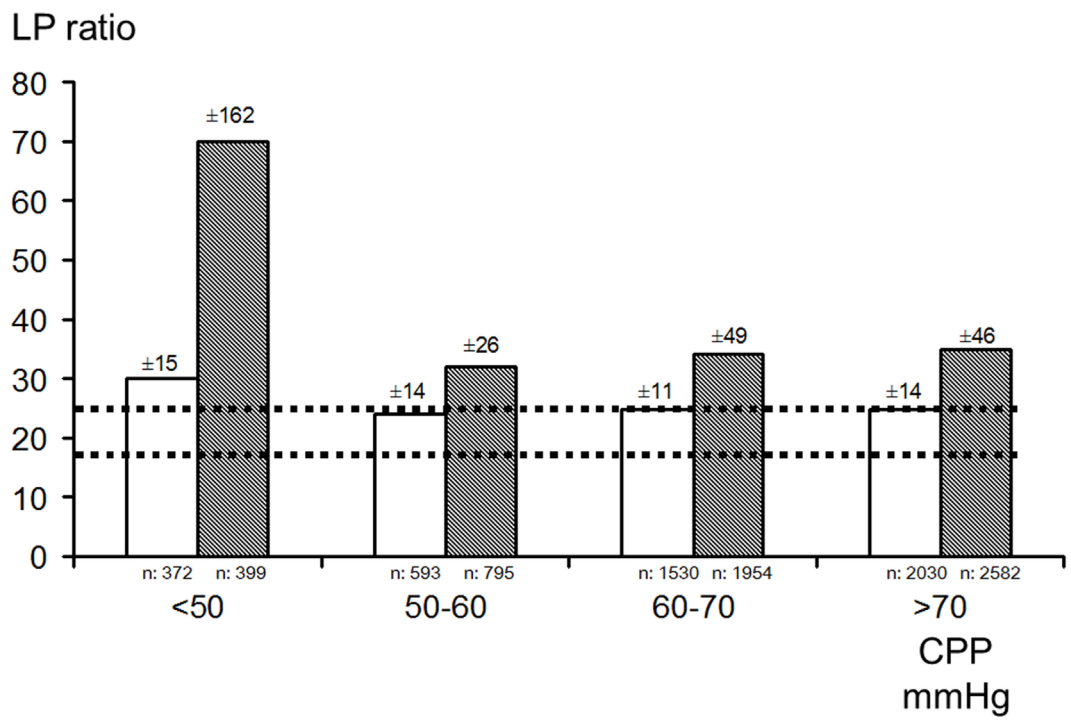


FIGURE 11 | Bar graphs demonstrating mean (±SD) lactate/pyruvate (LP) ratio in the better (white bar) and worse (gray bar) microdialysis catheter positions in relation to four ranges of cerebral perfusion pressure (CPP) in patients with severe traumatic brain lesions. Interrupted lines indicate the range (mean ± SD) in healthy brains in humans during wakefulness. Data from Ref. (182, 194), respectively.

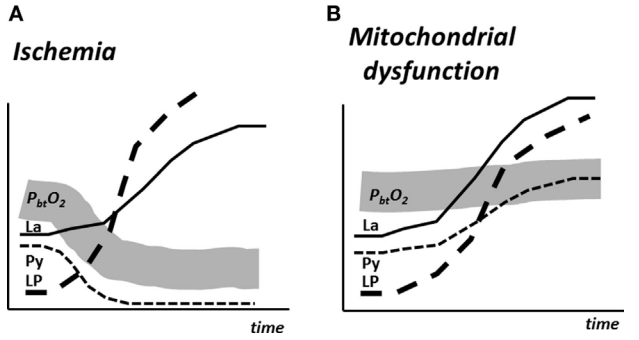


FIGURE 12 | Schematic illustration of cerebral tissue oxygenation ($P_{bt}O_2$) and changes in the levels of lactate (La), pyruvate (Py), and the lactate/pyruvate (LP) ratio in experimental cerebral ischemia **(A)** and mitochondrial dysfunction **(B)**. Data from Ref. (200).

presently under investigation. One example is cyclosporine A, which is thought to decrease mitochondrial damage by blocking opening of the mitochondrial permeability transition pore (202). The protective effect of cyclosporine in cerebral ischemia was in 1995 described in experimental studies (203). However, its clinical usefulness has not yet been documented. Irrespective of the mechanisms underlying mitochondrial dysfunction, a beneficial therapeutic intervention would probably be reflected in normalization of the biochemical variables analyzed and displayed at the bedside.

Lactate Supplementation for Treatment of TBI Patients

Based on association with the so-called astrocyte-to-neuron lactate (ANL) shuttling hypothesis, a number of publications have suggested the use of lactate as supplemental brain fuel after TBI. The ANL shuttle model was based on an observed glutamate-evoked release of lactate in cultured astrocytes indicating that glycolysis provides the ATP needed for astrocytic uptake of neurotransmitter glutamate and its conversion to glutamine and the assumption that the released lactate is oxidized by nearby neurons (204). However, ANL shuttling has never been quantified and validated as being metabolically significant in living brain, the cellular source(s) of lactate in brain have not been identified, and there are many independent lines of strong evidence against the lactate shuttle model. Accordingly, lactate shuttling and utilization has remained a controversial issue (205–212). *In vitro* studies have shown that lactate can maintain ATP levels but not sustain neuronal signaling (213–217). These observations underscore the critical roles of glucose metabolism upstream of pyruvate/lactate for neuronal function.

It is well documented and uncontroversial that lactate may serve as a supplemental brain fuel when its blood level is elevated and exceeds the intracerebral level for example during exercise or lactate infusion (218–220). However, it is important to note that the metabolic efficacy of lactate supplementation depends on functional integrity of mitochondria (211). This fact has sometimes been overlooked which may lead to unfortunate clinical decisions (221, 222). A prior assessment of oxidative capability in each patient would be required if the metabolic benefits of lactate were to be evaluated.

Furthermore, it is important to remember that lactate transport across the BBB is an example of facilitated diffusion. The transport does not directly require chemical energy from ATP hydrolysis but the lactate molecules move down their concentration gradient. Accordingly, net transport of lactate into the brain requires that blood lactate level is higher than its intracerebral concentration. Thus, the recent report that transport of lactate into the brain may occur against a concentration gradient in brain trauma patients is erroneous (223). The observation is fully explained by the imprecision of the biochemical analytical biochemical techniques used (189).

As discussed previously, the BBB has a very low permeability for sodium (cf. **Table 1**). Accordingly, infusion of hypertonic sodium lactate is usually effective in reducing a dangerous increase in ICP (224, 225). If, in a patient with compromised CBF due to high ICP, hyperosmolar therapy decreases ICP, improvement of cerebral energy metabolism would be expected and does not indicate a specific beneficial metabolic effect of lactate infusion. For example, in a series of patients with cerebral hemorrhage and ICP > 20 mmHg, infusion of mannitol (1 g/kg) resulted in a significant decrease in ICP and cerebral LP ratio, increased CPP, and unchanged $P_{bt}O_2$ and concentration of cerebral glucose (226). In a situation of compromised cerebral energy metabolism and an increase of LP ratio, lactate flooding is associated with considerable risk. For example, it may inhibit glycolytic and pentose shunt fluxes in neurons and astrocytes and impair glycogenolysis in astrocytes (212).

Bedside Evaluation of Cerebral Energy State—Future Possibilities

As the microdialysis probe reflects the biochemistry from a very narrow zone surrounding the dialysis membrane, appropriate positioning of the catheter in relation to focal lesions is necessary for a correct interpretation of the data obtained (193). During NCC, information regarding global cerebral energy state in addition to the regional information obtained from conventional intracerebral microdialysis would be valuable. Such information would also be of importance during critical care of other severe conditions when cerebral energy metabolism may be jeopardized without focal lesions (e.g., open-heart surgery, resuscitation after cardiac standstill, hemorrhagic or septic shock, toxic states). However, in these conditions, it is for various reasons difficult or impossible to insert intracerebral catheters. An alternative technique that avoids the penetration of cerebral tissue and still gives continuous bedside information regarding global cerebral energy state would be of apparent interest.

In a recent experimental study of induced hemorrhagic shock, the LP ratio in the draining cerebral vein (superior sagittal sinus) was compared to the LP ratio obtained from simultaneous intracerebral and intra-arterial microdialysis (227). In patients undergoing open heart surgery with cardio-pulmonary by-pass (CPB), a similar technique has been presented. The LP ratio obtained from microdialysis of the internal jugular vein increased significantly during CPB, indicating compromised cerebral oxidative metabolism, while conventional monitoring by NIRS did

not show a corresponding decrease in cerebral oxygenation (228). Future studies will show whether the LP ratio obtained from draining cerebral venous blood will be informative enough to be used as a method to evaluate cerebral global energy state bedside.

Presently, the microdialysis technique used in clinical routine does not permit online monitoring. Today, the technique is laborious since it is necessary for the NCC personnel to transfer microvials at regular time intervals from the microdialysis catheter to the bedside analyzer. Techniques utilizing biosensors for true online monitoring are under development (229). By utilizing these techniques, it will probably in the near future be possible to monitor glucose, lactate, and pyruvate online bedside during NCC. If the LP ratio obtained from microdialysis of cerebral venous blood gives useful information regarding global cerebral redox state, we might in the near future have the possibility to monitor global cerebral energy state online. The technique might be used not exclusively during NCC but also during general intensive care where insertion of an intracerebral catheter is contraindicated or impossible.

Summary: Cerebral Energy Metabolism

Since decades, the biochemistry of cerebral energy metabolism has been studied and documented in animal experiments. The microdialysis technique permits bedside monitoring of the most important biochemical variables during NCC. For a correct interpretation of the data, it is important to be aware of the prerequisites and limitations of the micordialysis technique and the biochemical analyses. The information obtained gives information regarding cerebral energy state and can be used to separate cerebral ischemia from mitochondrial dysfunction. However, the biochemical data obtained by intracerebral microdialysis are representative of a very small volume of tissue. Accordingly, a correlation to clinical outcome would be expected only when the volume studied is representative of a relatively large part of the brain. Future routine bedside monitoring of cerebral energy metabolism will probably depend on whether techniques permitting true online biochemical analysis by sensors are presented.

CONCLUDING REMARKS

Neurocritical care is especially focused on problems related to ICP, CBF, and cerebral energy metabolism. Specific monitoring techniques have been introduced to give information regarding variables within these areas. To be of real importance for NCC, the techniques must give data that are obtained frequently/continuously, presented bedside and included in the clinical decision making. The information presented may be representative of a large part of the brain (global technique) or a very small volume surrounding the probe (focal technique). Both techniques have specific advantages and disadvantages. Many clinical studies have been devoted to examine the relation between the data obtained and clinical outcome. These efforts are not always motivated and may be questioned for several reasons. Most importantly, though physiological and biochemical data obtained by a global technique might be related to outcome, it is not justified to use data from a focal technique in this way unless it is known that these data are representative of a large part of the brain. Other clinical studies have examined possible correlations between the different physiological and biochemical techniques. Such correlations may be justified provided it is known that there is a physiological or biochemical relation between the variables. We will here briefly summarize our opinions regarding some of the techniques discussed previously.

Global Physiological Techniques

Intracranial pressure is a keystone within NCC and CPP is calculated from ICP and the simultaneously monitored MAP. To be of clinical importance, ICP must be measured accurately and displayed continuously at the bedside. Presently, this may be achieved in two ways: from an intra-ventricular catheter or from a pressure-sensitive sensor placed in the brain.

The index named PRx is not related to a well-defined physiological mechanism or process, and the data obtained should not be regarded as a definite measure of vascular resistance or autoregulation. Several clinical studies have tried to correlate PRx with $PbtO_2$ as well as with biochemical data obtained from microdialysis. As the mechanism behind PRx is not defined it is unclear why this global variable should be expected to correlate with any of these two focal techniques.

Under physiologically stable conditions, $SjvO_2$ is interpreted as reflecting the balance between cerebral oxygen delivery and the cerebral metabolic rate of oxygen. However, the normal range of $SjvO_2$ remains controversial, it provides limited information in patients with focal lesion, and technical monitoring problems are common. For obvious reasons, the correlation between $SjvO_2$ and $PbtO_2$ is often poor.

Focal Physiological Technique

$PbtO_2$ gives online information regarding tissue oxygen tension which is determined by the blood flow, the blood oxygen tension, and oxygen diffusion through the tissue. However, it does not disclose whether this oxygen tension is sufficient for maintaining adequate metabolism, and it has not been possible to define a threshold for $PbtO_2$ below which hypoxic/ischemic cerebral damage occurs. As $PbtO_2$ monitoring is a focal technique, a correlation to clinical outcome would be expected only when the data obtained reflects the situation in a relatively large part of the brain. The prerequisites for expecting a correlation between $PbtO_2$ and LP ratio is discussed in the following paragraph.

Focal Biochemical Technique

Presently, intracerebral biochemistry can only be evaluated bedside by utilizing microdialysis. During NCC routine, biochemical analyses can be used to give bedside information on cerebral energy status and signs of acute cellular degradation. In clinical praxis, it is important to use the technique in a standardized fashion which allows comparison between the data obtained and the levels observed in normal human brain. Routine biochemical analysis allows a clinically important separation of cerebral ischemia and mitochondrial dysfunction. The biochemical information obtained is representative of a very small tissue volume and a correlation to clinical outcome would be expected only when the conditions in this volume is representative of large part

of the brain. As the LP ratio reflects cytoplasmatic redox state, a correlation to $PbtO_2$ would be expected during cerebral ischemia but not during mitochondrial dysfunction.

Global Biochemical Techniques

Presently, there is no technique available for evaluation of global cerebral energy state during NCC. Intraventricular insertion of the microdialysis catheter might be used in combination with conventional biochemical analysis, but the clinical benefits of this procedure have so far not been explored. Whether the LP ratio obtained from the draining cerebral venous blood might be informative is presently studied under experimental and clinical conditions. If the technique can be shown to give information regarding "global cerebral redox state," it might be used to evaluate cerebral energy state also in clinical situations where insertion of an intracerebral catheter is risky or impossible.

General Considerations for the Future

Future progress within NCC will necessitate a close collaboration between clinicians, experimental laboratories and companies developing new products for bedside monitoring and analysis. All physiological and biochemical interpretation should be based on solid data obtained in controlled experimental studies. It is important to realize that new biochemical principles will not be discovered under clinical conditions by utilizing routine bedside analytical techniques. For the intensivist working within NCC, it is already today a demanding task to interpret the interrelated and complex physiological and biochemical variables displayed bedside. In the future, this complexity will probably increase. To meet this development, the future intensivist will need deeper theoretical knowledge and, ideally, experiences from experimental studies within these specific areas of importance. Finally, the clinicians utilizing advanced monitoring techniques must understand the underlying principles and the limits and pitfalls associated with each specific method.

AUTHOR CONTRIBUTIONS

Conception and design of the review; drafting the work and revising the review critically for important intellectual content; final approval of the version to be published; agreement to be accountable for all aspects of the work ensuring that questions related to the accuracy or integrity of any part of the work are appropriately presented (C-HN, L-OK, and MO).

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REVIEW
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Critical Evaluation of the Lund Concept for Treatment of Severe Traumatic Head Injury, 25 Years after Its Introduction

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When introduced in 1992, the Lund concept (LC) was the first complete guideline for treatment of severe traumatic brain injury (s-TBI). It was a theoretical approach, based mainly on general physiological principles—i.e., of brain volume control and optimization of brain perfusion and oxygenation of the penumbra zone. The concept gave relatively strict outlines for cerebral perfusion pressure, fluid therapy, ventilation, sedation, nutrition, the use of vasopressors, and osmotherapy. The LC strives for treatment of the patho-physiological mechanisms behind symptoms rather than just treating the symptoms. The treatment is standardized, with less need for individualization. Alternative guidelines published a few years later (e.g., the Brain Trauma Foundation guidelines and European guidelines) were mainly based on meta-analytic approaches from clinical outcome studies and to some extent from systematic reviews. When introduced, they differed extensively from the LC. We still lack any large randomized outcome study comparing the whole concept of BTF guidelines with other guidelines including the LC. From that point of view, there is limited clinical evidence favoring any of the s-TBI guidelines used today. In principle, the LC has not been changed since its introduction. Some components of the alternative guidelines have approached those in the LC. In this review, I discuss some important principles of brain hemodynamics that have been lodestars during formulation of the LC. Aspects of ventilation, nutrition, and temperature control are also discussed. I critically evaluate the most important components of the LC 25 years after its introduction, based on hemodynamic principles and on the results of own an others experimental and human studies that have been published since then.

Keywords: brain injury, intracranial monitoring, neuroinflammation, neuroradiology, neuro-intensive care, the Lund concept, the penumbra zone, brain perfusion

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INTRODUCTION

The principles of the Lund concept (LC) for treatment of severe traumatic brain injury (s-TBI) (Glascow Coma Score 3–8) were introduced in 1992 at the Swedish Society of Anesthesia congress in Lund (1), with a first clinical study published in 1994 (2). It was a new, controversial approach, mainly based on physiological and pathophysiological principles of brain volume regulation and perfusion of the most injured parts of the brain. The experience from 25 years of use supports the

view that the LC appears to be beneficial not only for the brain but also for other organs of the body, such as the lung. Treatment with the LC is primarily based on the pathophysiological mechanisms behind symptoms rather than just treating the symptoms. A comprehensive presentation of the principles and guidelines of the LC has already been published (3). It is reasonable to assume that making the hypoxic penumbra zone of the injured brain to survive by optimizing brain perfusion and oxygenation is crucial when discussing outcome of the brain. Thus, apart from counteracting an increase in intracranial pressure (ICP), the LC is also aimed at improving microcirculation and oxygenation of the hypoxic penumbra zone and can, therefore, be classified as an *ICP and perfusion-targeted therapy*.

In principle, the current concept does not differ much from the one originally presented, except that the venoconstrictor dihydroergotamine has been phased out. Dihydroergotamine was initially used in patients with a refractory, life-threatening high ICP, but its use was discontinued because of potential adverse peripheral vasoconstrictor effects.

The LC resulted in treatments that appeared to be quite contrary to the alternative ideas presented a few years later. Alternative guidelines for treatment of s-TBI, e.g., the Brain Trauma Foundation guidelines and European guidelines (4, 5), were mainly based on meta-analytic approaches and systematic reviews, originating from clinical outcome studies. The LC was severely criticized by advocates of the alternative guidelines, but in the intervening 25 years the LC has gained more acceptance. Continuous ICP recording is recommended in both the LC and the alternative s-TBI guidelines.

The LC differs from other guidelines not only regarding therapeutic components but also regarding the time to start the therapy. In the alternative guidelines, the ICP-reducing therapy should start when ICP is above 20 mmHg (4, 6), a value that has been increased to 22 mmHg in the latest update from the Brain Trauma Foundation (7). In contrast, the LC recommends that the therapy should start early after arrival at the hospital irrespective of prevailing ICP (3, 8). This will counteract an increase in ICP prophylactically from the start.

Even though the LC on the one hand and the alternative guidelines on the other still differ from each other in essential respects, the Brain Trauma Foundation guidelines have moved closer to the LC in the past 10–15 years, e.g., concerning cerebral perfusion pressure (CPP) and more strict use of vasopressors and mannitol (6) (**Table 1**).

Several smaller, single-center outcome studies using the LC have shown good outcome (9–11). Two smaller randomized studies compared a modified version of the LC with a more conventional CPP-targeted treatment (12, 13). Both of these studies showed significantly better outcome with the modified LC than with the conventional guideline. All outcome studies using the LC have been summarized and discussed in a recent review (8).

A study by Patel et al. (14) evaluated trends in outcome in head-injured patients who underwent conventional treatments in England and Wales between 1989 and 2003. They found no improvement in outcome during that period. This conclusion has been supported by two recent reviews (15, 16), which showed that modern research has not improved outcome following TBI, although progress has been made in understanding the pathophysiology of s-TBI and the general hospital care has been improved. A study of s-TBI patients, in which the patients were treated according to the Brain Trauma Foundation guidelines, did, however, show improvement in outcome between 2001 and 2009 (17).

TABLE 1 | Examples of components where the BTF guideline has approached or deviated from the Lund concept (LC).

LC	BTF at its introduction (1996)	BTF 2007/2016
Cerebral perfusion pressure (CPP) 50–70 mmHg	CPP above 70 mmHg	CPP 50–70 mmHg
Avoidance of osmotherapy	Osmotherapy a main intracranial pressure-reducing therapy	Osmotherapy still used, but with more caution
Avoidance of vasopressors	High doses of vasopressors accepted to keep CPP above 70 mmHg	Vasopressors can be used, but less frequent to avoid ARDS
Active cooling is not used	Active cooling is accepted	Active cooling is not used
Albumin recommended as plasma volume expander	Albumin recommended as plasma volume expander	Albumin not specifically recommended

Apparently, we still lack convincing evidence-based support for many of the treatments used in various s-TBI guidelines, and this in spite of the fact that several randomized studies have been performed evaluating specific components (14, 15), such as effect of hypothermia, or any of the pharmacological treatments tested. Further, there is no large randomized study performed comparing the overall outcome from different s-TBI guidelines. From this point of view, all guidelines are equally deficient. A specific therapy, therefore, to a large extent must be based on other types of input such as smaller clinical outcome studies, experimental studies, basal physiological principles, systematic reviews, and meta-analytic approaches.

To understand and evaluate the different components of the LC, one must be familiar with its theoretical background. In the present review, I describe some basic physiological and pathophysiological mechanisms controlling brain volume and brain perfusion, which have been lodstars during formulation of the LC. "Nature knows best" is a motto of the LC, and it strives for normality for most hemodynamic and ventilatory parameters—as well as normality regarding electrolytes, temperature, nutrition, and stress. Experimental and human studies from our group and other groups evaluating the different components of the LC will be presented in this review, and used for a critical evaluation of the therapeutic components of the LC. I also speculate about possible not proven physiological explanations of some well-known, but still not fully understood, scenarios in s-TBI patients.

PRINCIPLES FOR BRAIN VOLUME REGULATION

As the brain is enclosed in a rigid cranium with only minor space for intracranial expansion, the brain volume must be kept at a

relatively constant level to avoid adverse alterations in ICP. The normal brain, therefore, has a more sophisticated volume regulation than that of other organs of the body.

In all organs outside the brain, the capillaries are passively permeable to smaller molecules such as Na^+ and Cl^- ions, and to some extent also to larger macromolecules, such as proteins. The capillaries of the normal brain differ from those of the rest of the body, in the sense that they are passively permeable to water only, a feature that characterizes the intact blood–brain barrier (BBB) (**Figure 1A**) (18). Electrolytes and larger molecules cannot cross the intact BBB in a passive way. In the injured brain, on the other hand, especially in the most injured parts of the brain, the BBB is disrupted, which means that electrolytes (but not larger molecules) can pass passively (**Figure 1B**). Thus, the passive permeability to proteins and other macromolecules of cerebral capillaries in both the normal and the injured brain is very low, as indicated by the small increase in protein concentration in cerebrospinal fluid (CSF) of only 0.5–2 g/L from values of 0.5–1 g/L after an s-TBI, as compared to a normal protein concentration in plasma of 60–70 g/L.

An imbalance of the Starling fluid equilibrium in the brain—in terms of an increase in transcapillary hydrostatic capillary pressure, e.g., after an increase in arterial pressure, or a decrease in transcapillary oncotic pressure—will start filtration (**Figure 1A**). If the BBB is intact, the filtrate consists only of water. This means that the filtration will cease very soon, as water filtered to the interstitium decreases the interstitial crystalloid osmotic pressure by dilution from its normal value of about 5,500 mmHg, creating an absorbing crystalloid osmotic counter-pressure. This explains why an intact BBB is essential to maintain brain volume at a relatively constant level (18).

A disrupted BBB, on the other hand, means that following an imbalance in the Starling fluid equilibrium toward filtration, the filtration will continue, as the filtrate has about the same crystalloid composition as the interstitium (**Figure 1B**). Thus, the filtrate will cause no or just a small amount of interstitial osmotic dilution, and no or just a small amount of absorbing osmotic counter-pressure will develop. The filtration will, therefore, continue, creating a vasogenic brain edema, until it is successively counteracted and stopped by the subsequent increase in ICP. According to these principles, the vasogenic brain edema can develop only if there is an imbalance in the Starling fluid equilibrium and a simultaneous passive permeability to small solutes (e.g., Na^+, Cl^-).

Because of impaired autoregulatory capacity in the injured brain, partly due to impaired myogenic capacity, there is an increase in hydrostatic capillary pressure, which will be gained by an increase in the arterial inflow pressure. Realistic values of the imbalance in the Starling equation created by the increase in transcapillary hydrostatic pressure when autoregulation is impaired can be estimated to be 4–5 mmHg at most.

At first sight, it seems paradoxical that the vasogenic brain edema can result in a much higher increase in ICP than the initial increase in transcapillary hydrostatic capillary pressure. This "*paradoxical event*," however, can be explained from hemodynamic principles for an organ enclosed in a rigid shell and will be discussed in more detail below under Section "Hemodynamic Consequences of the Rigid Cranium."

Most likely, various permeability-increasing proinflammatory substances released after a head trauma are involved in the disruption of the BBB. While a disrupted BBB is essential for the development of a vasogenic extracellular brain edema, the cytotoxic brain edema is mainly intracellular—caused by damage to cell membranes, e.g., from hypoxia and various toxic substances, such as cytokines and free radicals (19, 20). Magnetic resonance imaging has shown that intracellular edema is part of post-traumatic brain swelling, and occurs mainly around contusions (21). Mitocondrial dysfunction is also suggested as an important pathophysiological mechanism after an s-TBI. Several proinflammatory substances released after brain trauma have been evaluated as substances responsible for development of cell injury and brain edema (22). All studies so far that have tested antagonists

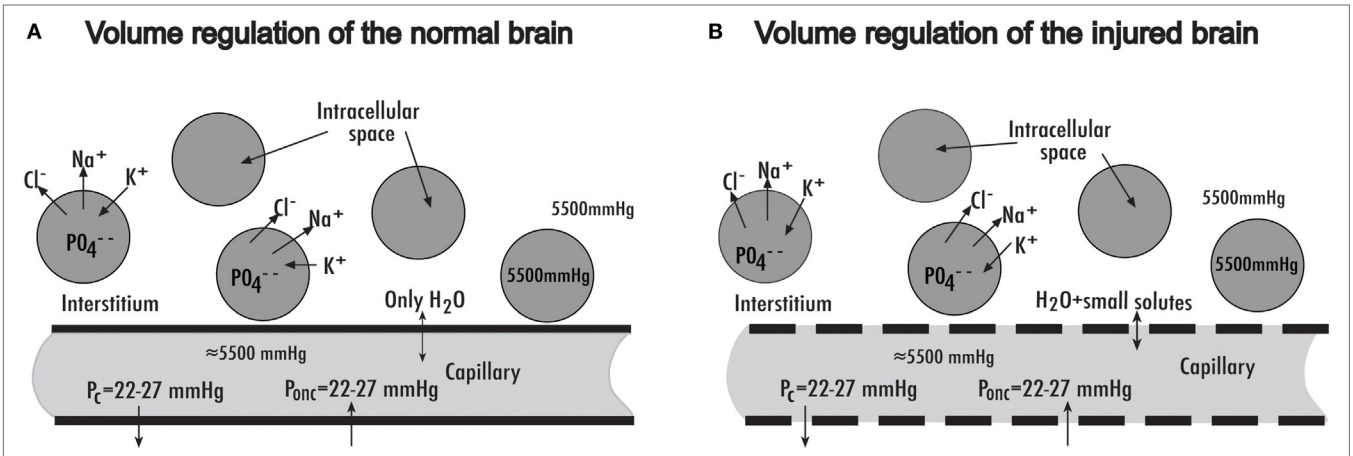

FIGURE 1 | **(A)** Schematic illustration of a cerebral capillary in the normal brain with intact blood–brain barrier (BBB), also showing the Starling forces (transcapillary hydrostatic and oncotic pressures) responsible for transcapillary fluid exchange. Only water can pass through the intact BBB passively. **(B)** Cerebral capillary and the Starling forces in the injured brain with disrupted BBB, in which the capillaries are passively permeable to water and small solutes. For more details of the volume regulation mechanisms in the normal and injured brain, see text. Reproduced from Ref. (3), with permission.

of some of the toxic substances discussed, have, however, failed to improve outcome (22–24).

Irrespective of types of substances released, hypoxia is presumed to be an important triggering mechanism and cause of secondary injury to the brain (25, 26). As the most injured parts of the brain suffer from severe hypoxia, improvement of perfusion and oxygenation of these areas should help to reduce the cytotoxic brain edema, a hypothesis adopted in the LC. Hypoxia may also increase brain edema by increased interstitial osmotic pressure from cellular and molecular disintegration inducing transcapillary filtration (27). Consequently, the main parts of both the vasogenic and the cytotoxic brain edema will develop in the most injured parts of the brain.

HEMODYNAMIC CONSEQUENCES OF THE RIGID CRANIUM

Important hemodynamic characteristics of the brain result from the fact that it is enclosed in a rigid cranium. Some of these characteristics will be discussed below from the schematic illustration of the brain circulation enclosed in a rigid shell shown in **Figure 2**.

Intracranial pressure for the normal brain is 8–11 mmHg, and it is kept at this level by a balance between production and consumption of CSF. This can be compared with normal tissue pressure in the rest of the body of 0 to −2 mmHg (28). The brain is the only organ of the body with a significantly positive tissue pressure. This is not a coincidence, as a positive ICP is essential for proper function of the normal brain, as explained below.

The venous pressure outside the dura (P_V in **Figure 2**) is close to 0 or even negative at upright position. This means that there is a pressure fall in the veins between the subdural and the extradural space. This pressure fall has—somewhat inappropriately—been called a waterfall phenomenon (29–32). As early as 1928, it was shown experimentally (33), that this pressure fall creates a venous collapse at a short distance before the veins leave the brain, creating a subdural venous outflow vascular resistance (R_{out} in **Figure 2**). As venous pressure just before R_{out} (P_{out} in **Figure 2**) will change in parallel with the variation in ICP, the resistance created by the passive collapse (R_{out}) is directly related to the pressure fall ICP-P_V (**Figure 2**) (31, 34).

The existence and hemodynamic consequences of a variable collapse for an organ enclosed in a rigid shell was confirmed and analyzed experimentally on a normally perfused cat skeletal muscle enclosed in a closed plethysmograph (30, 31). It is relevant to simulate hemodynamics of the brain related to the rigid cranium on a skeletal muscle enclosed in a plethysmograph, as the results are general for any organ enclosed in a rigid and closed shell (30–32).

Figure 3 shows some hemodynamic parameters from such an experimental model. As seen, $P_{orifice}$ (corresponding to P_{out} in **Figure 2**), tissue volume (Vol), and blood flow to the organ (Q) do not change when venous pressure (P_V in **Figure 3**, corresponding to P_V in **Figure 2**) varies, as long as P_V is below the tissue pressure (P_{tissue}) of 20 mmHg. An increase in P_V when P_V is above P_{tissue} (there is no protecting venous collapse) results in an increase in $P_{orifice}$ (P_{out} in **Figure 2**), an increase in hydrostatic capillary pressure (P_c) and increase in tissue volume (Vol), while blood flow (Q) is reduced due to reduction in perfusion pressure. That P_c increases in parallel with an increase in P_{tissue} is supported by **Figure 3**—showing a P_c of about 34 mmHg at a P_{tissue} of 20 mmHg compared to a normal P_c in a skeletal muscle of 15 mmHg. It can be concluded from **Figure 3** that the variable passive venous collapse will protect the brain from extracranial venous pressure (P_V) variations as long as P_V is lower than ICP.

The existence of a passive sudural venous collapse and its hemodynamic consequences also finds indirect but strong support, for two reasons. First, what would happen in our daily lives if a subdural venous resistance protecting the brain from venous pressure variations did not exist? If this was the case, there would be a drastic reduction in intracranial venous blood volume (70–80% of the intracranial blood volume is situated on the venous side of the brain), with marked hemodynamic consequences

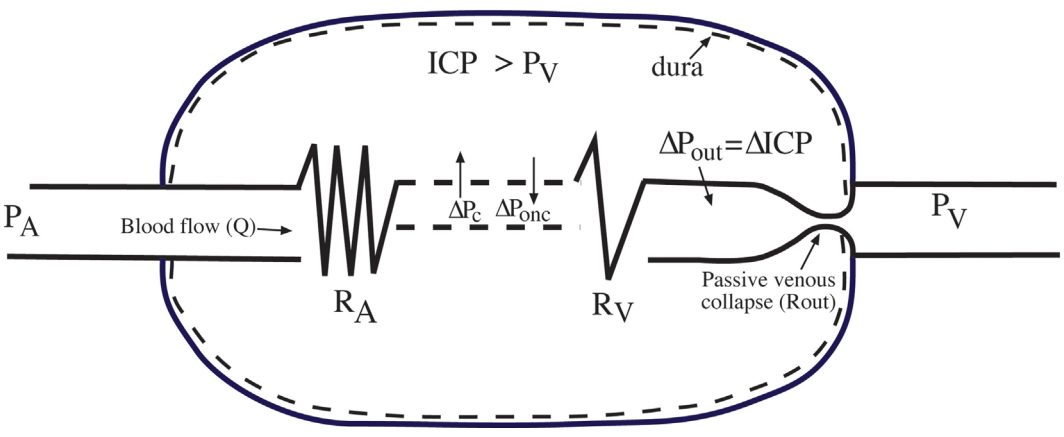

FIGURE 2 | Hemodynamic consequences for the brain enclosed in the rigid dura/cranium. ΔPc, transcapillary hydrostatic capillary pressure; ΔP_{onc}, transcapillary oncotic pressure; P_A, the arterial inflow pressure; Q, cerebral blood flow; R_A, arterial precapillary resistance; R_V, venular resistance; ΔP_{out}, transvascular pressure retrogradely to the subdural venous collapse (R_{out}); P_V, is extracranial venous pressure. For details, see text. Reproduced from Ref. (3), with permission.

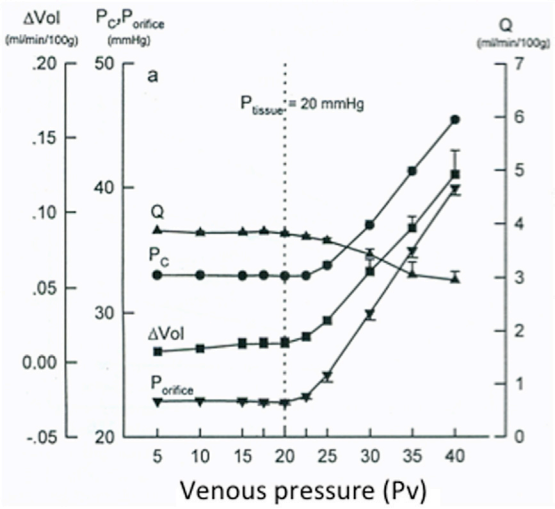

FIGURE 3 | Hemodynamic effects of an organ (skeletal muscle) enclosed in a rigid shell (plethysmograph) with a tissue pressure of 20 mmHg of variation in venous pressure just outside the shell (P_V). The experiment was intended to simulate the attention: hemodynamic effects of extradural P_V variations in the brain enclosed in the dura/cranium. The figure shows effects of change in P_V on hydrostatic capillary pressure (*P*c), on venous pressure just upstream of the venous collapse ($P_{orifice} = P_{out}$ in **Figure 2**), on change in tissue volume (ΔVol), and on blood flow (Q). Note that there are no hemodynamic effects of change in P_V if P_V is below the tissue pressure. If P_V is above tissue pressure, there is no protective venous collapse (no R_{out}, **Figure 2**), and the variations in P_V are transferred in retrograde manner to the skeletal muscle. Reproduced from Ref. (30), with permission.

when changing from supine position to upright position and *vice versa*. An extracranial venous collapse when changing from supine to upright position might also help to protect the brain from variations in venous pressure.

Second, calculation of CPP as arterial pressure (P_A) minus ICP and not minus P_V, as done when calculating the perfusion pressure in other organs of the body, can be entirely explained by the existence of a variable passive subdural outflow resistance (R_{out}). Thus, CPP is P_A minus the pressure just upstream of R_{out}, which is the same as ICP (**Figure 2**). This means that by accepting CPP as $P_A - ICP$, we also have to accept a passive variable subdural venous resistance compensating for extradural variations in P_V (**Figures 2** and **3**). Note that if P_V is higher than ICP, there is no protecting venous collapse and perfusion pressure should be calculated as $P_A - P_V$.

As mentioned under Section "Principles for Brain Volume Regulation" above, the increase in ICP from a vasogenic brain edema may be much greater than the disturbance in the Starling fluid equilibrium following an increase in hydrostatic capillary pressure and decrease in plasma oncotic pressure. This paradoxical event has been described previously (34) and will be commented on below.

Imbalance between hydrostatic and oncotic transcapillary pressures in the injured brain with a disrupted BBB will start filtration and slowly increase ICP. The simultaneous increase in R_{out} due to the increase in $ICP - P_V$ means a similar increase in P_{out}, which will be transferred in a retrograde manner to the capillaries (**Figure 2**). This will result in an increase in P_c, which will cause further filtration and further increase in ICP, and so on. A new steady state at a raised ICP will finally be established. Due to a fall in pressure in venules of about 20%, only about 80% of the increase in ICP is transferred in retrograde manner to the capillaries. This means that the highest increase in ICP by a vasogenic brain edema at steady state will be eight times larger than the initial imbalance between transcapillary hydrostatic pressure (P_c) and the transcapillary oncotic pressure (P_{onc}) (34). This mechanism explains why the increase in ICP caused by vasogenic brain edema can be much larger than the initial increase in P_c and decrease in P_{onc}.

Consequently, a reduction in P_c—for example, by antihypertensive therapy—may reduce ICP caused by a vasogenic edema at most by eight times more than the initial decrease in P_c. This physiological scenario strengthens the motivation for using antihypertensive therapy in head-injured patients with raised ICP (see under "Blood pressure" below).

The hypothesis of a much greater increase in ICP than the initial imbalance in the Starling equilibrium has found support in a study on the cat (35), the results of which are presented in **Figure 4**. It showed, as expected, that ICP was not affected by an increase in mean arterial pressure of 30 mmHg by infusion of angiotensin II and dobutamin when the BBB was intact (**Figure 4A**). When the BBB was disrupted, accomplished by intrathecal endotoxin infusion, there was an increase in ICP of 25 mmHg at steady state 5 h after a similar increase in arterial pressure of 30 mmHg (**Figure 4B**). The marked increase in ICP in this study supports the importance of the blood pressure for development of a vasogenic brain edema at a disrupted BBB and also the hypothesis that ICP can increase much more than the initial increase in P_c.

Some clinical consequences for the brain of the existence of a variable passive subdural venous collapse will be presented below. For example, the decrease in extracranial venous pressure by head elevation causes a corresponding increase in R_{out}, preventing the extradural venous pressure decrease from being transferred in retrograde manner to the brain (31). There will, therefore, be no increase in venous drainage from the brain (i.e., no decrease in intracranial venous blood volume) after head elevation, as has also been suggested previously (36, 37) (cf. **Figure 3**). The immediate decrease in ICP after head elevation can instead be explained by passive decrease in intracranial blood volume on the arterial side, when arterial pressure to the brain is reduced.

Most probably due to the suggested risk of increase in venous pressure and ICP by positive end-expiratory pressure (PEEP), PEEP is not recommended as an obligatory therapy in alternative guidelines (5–7). The physiological theories presented above, however, contradict the idea of such a risk—as the increase in extracranial venous pressure by PEEP will not be transferred to the brain. This hypothesis found support in a clinical study by Caricato et al., which showed that PEEP increased central venous pressure and jugular pressure without affecting ICP (38). Thus, PEEP (normally 6–8 cmH_2O) has been mandatory in the LC to protect the lung from atelectasis and ARDS. PEEP should always be below ICP to be sure that the subdural outflow resistance (R_{out}

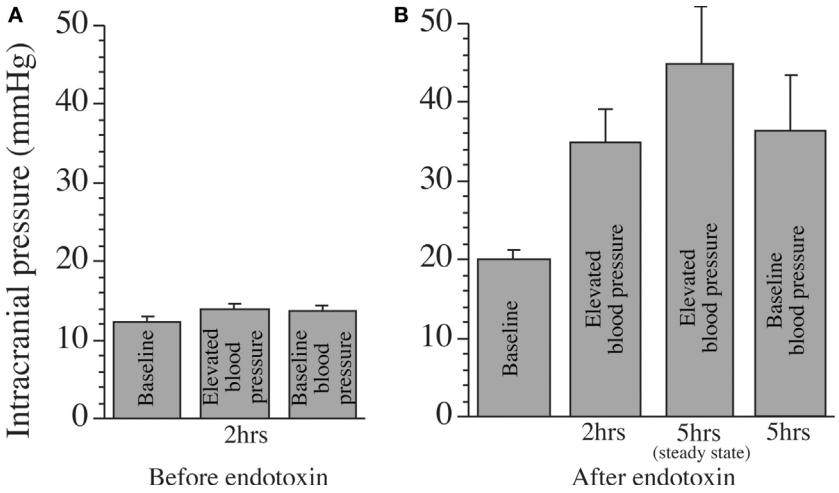

FIGURE 4 | Effects of an increase in mean arterial blood pressure of 30 mmHg, by intravenous infusion of dobutamin and angiotensin II, on intracranial pressure (ICP) in the cat. **(A)** ICP with intact blood–brain barrier (BBB) with normal arterial blood pressure (baseline) and after 2 h of elevated blood pressure (by about 30 mmHg). ICP was not affected by the increase in blood pressure after 2 h and after 5 h (5-h data are not shown). There was no change in ICP when mean arterial pressure returned to baseline. **(B)** ICP after disruption of the BBB with intrathecal endotoxin infusion at normal arterial blood pressure (baseline) and after 2 and 5 h with increased mean arterial blood pressure by about 30 mmHg. There was a significant increase in ICP by about 25 mmHg from the increase in arterial pressure. ICP was slightly reduced shortly after the arterial blood pressure returned to baseline, an effect most likely due to a decrease in intracranial blood volume. As can be seen, this effect was smaller from a normal ICP **(A)** than from a higher ICP **(B)**, due to the unlinear pressure–volume curve for the brain. Reproduced from Ref. (35), with permission.

in **Figure 2**) is preserved. By now results of several studies have supported the use of PEEP in TBI patients (38–41).

The normal brain is protected from variations in arterial pressure through a balance between the active myogenic and the metabolic control systems, a phenomenon called autoregulation. The myogenic control system is vulnerable, which explains why the true autoregulation of blood flow is significantly depressed after a brain trauma. The degree of autoregulation for the whole brain is also affected negatively by the fact that blood flow in the contusion areas is very low and that these areas lack autoregulation. Impaired autoregulation in less injured parts of the brain, mainly through impared myogenic reactivity, is probably of less importance for outcome—as impaired myogenic reactivity means vasodilation.

In summary, the normal brain is protected from arterial pressure variations *via* a myogenically active mechanism called autoregulation of blood flow, which is depressed after a head injury. Both the normal and the injured brain are protected from venous pressure variations *via* a passive mechanism based on the existence of a variable venous outflow resistance.

BLOOD PRESSURE AND CPP

Hypertension is common after a head injury, most likely due to a hyperadrenergic state (42–45). If arterial blood pressure is low after an s-TBI without external bleeding, in most cases it is an effect of hypovolemia. The purpose of maintaining a high CPP in the alternative guidelines was to prevent cerebral ischemia by squeezing oxygenated blood through the swollen brain (CPP-targeted therapy) (4, 5, 46). This has been a generally accepted concept in most of the alternative guidelines. A human study by Simard and Bellefleur (47) and a rabbit study by Durward et al. (48) raised questions regarding this concept.

A supranormal CPP was criticized in the LC from the start, based on physiological aspects of brain volume regulation of the injured brain as described above and also by adverse effects of vasopressors. Thus, a main component of the LC is the use of antihypertensive therapy with the purpose of reducing arterial pressure, cerebral hydrostatic capillary pressure, and adrenergic stress, which are all elevated after a head injury. Only antihypertensive drugs that do not induce a simultaneous cerebral vasodilation are used, as cerebral vasodilation will increase hydrostatic capillary pressure, intracranial blood volume, and ICP. If a marked brain edema and high ICP have already developed, antihypertensive therapy will reduce brain edema, but this is a slow process, most likely due to a relatively low water permeability in the brain. The antihypertensive therapy needs time to act, and it may take hours or even a day before the raised ICP shows signs of reduction after the start of antihypertensive therapy. It is, therefore, better to counteract an increase in ICP early by starting the ICP-reducing therapy as soon as possible after arrival at the hospital, regardless of the prevailing ICP, as recommended in the LC (3, 8).

The antihypertensive mechanisms originally used were beta-1 blockade and alpha-2 agonist. If not sufficiently effective, they were complemented with an angiotensin II antagonist (see below). The patient should normally be in a flat position with one pillow under the head. However, if there is a need to reduce CPP more than can be obtained from the antihypertensive treatment, the LC accepts moderate head elevation (15–20°). During calculation of CPP after head elevation, one must compensate for the increased vertical distance between the head and the heart.

Antihypertensive treatment was strongly questioned when introduced with the LC in 1992. It was entirely contrary to the general recommendations to keep CPP above 70 mmHg with vasopressors (4, 5, 46). This recommendation was changed in the Brain Trauma Foundation guidelines from 2007 (6) after publication of a study that showed that outcome was better with a relatively low CPP than with a high CPP, often obtained using vasopressors (49). It was later discussed whether the better outcome was an effect of the lower CPP or of less use of vasopressors, resulting in less ARDS (50).

Independently of this, US guidelines changed their recommendations in adults from a CPP of above 70 mmHg to a CPP of 50–70 mmHg (6), which is in the same range as has been recommended in the LC (3) and later also recommended in a study by Johnson et al. (51). A study by Elf et al. suggested that a CPP of 50–60 mmHg is acceptable if an optimal fluid therapy is used (52). Recommended CPP values in children are lower (3, 8). Note that CPP remains in the range of 60–70 mmHg with the LC in most adult patients in spite of the antihypertensive treatment, but somewhat lower CPP values than these can be accepted.

A CPP value in an unconscious patient after a TBI can be lower than what is normal in a healthy human being who is awake, especially if vasoconstrictors are not used. Just by lying in the supine position means that mean arterial pressure can be 15–20 mmHg lower than in upright position with the same perfusion, and even lower if the patient is sedated—as in the LC. In light of these considerations, it is reasonable that a CPP of 60–65 mmHg and even lower should be acceptable in s-TBI patients, if they are properly treated otherwise by avoiding hypovolemia, stress, and vasoconstrictors.

Note, however, that the CPP value alone does not reflect cerebral circulation, as it is also highly dependent on the blood volume status and the use of vasopressors and on the hyperadrenergic stress. Thus, a CPP of the lower range of 55–65 mmHg is only acceptable if normovolemia is maintained and no vasoconstrictors are used and with low adrenergic stress. Most CPP-targeted studies only present the CPP value without giving any information on blood volume and vasopressor status. This was the case also in those studies used in the latest Brain Trauma Foundation guidelines from 2016. In this version, the CPP recommendations have been changed from 50–70 mmHg to a lowest CPP of 60–70 mmHg (7).

As mentioned above, beta-1 blockade is an important antihypertensive component in the LC. It was initially strongly criticized by advocates of CPP-targeted guidelines. The criticism has more or less ceased after beta-blockade to head-injured patients received strong support from three independent human studies and one mouse study, which showed that beta-blockade was beneficial to the brain after s-TBI, with a significantly improved survival rate (53–56). Beta-blockade has also been shown to be protective on the cardiovascular system after TBI (57). We showed that beta-1 blockade has no direct influence on local cerebral hemodynamics after a TBI (58), and no side effects have been observed in TBI patients given beta-blockade. The LC is still the only TBI guideline to recommend beta-blockade.

It is well established that head-injured patients develop an adverse hyperadrenergic state with increase in proinflammatory sympathetic discharge and catecholamine release (42, 59). Alpha-2 agonists have become more and more popular as sedatives in general intensive care units and they damp a hyperadrenergic state as shown after cerebral ischemia (60). They are, however, still not recommended as a general drug for head-injured patients, except in the LC (3, 8, 9). Alpha-2 agonists effectively reduce blood pressure by their antisympathetic and sedative effects. They showed neuroprotective effects in an *in vitro* model of traumatic brain injury (61). An alpha-2 agonist decreases plasma catecholamine concentration and improves outcome from incomplete ischemia in the rat (62) and has no direct influence on local cerebral hemodynamics after TBI (58).

Most studies investigating the effects of alpha-2 agonists have analyzed the less selective catapressan. There are reasons to believe, however, that the newer, more selective alpha-2 agonist dexmedetomidine at a dose of 0.5–1.5 μg/kg/h is a better choice by minimizing vasoconsstrictor effects of a simultaneous alpha-1 stimulation. Note that much higher doses than those recommended in the LC both for catapressan and dexmedetomidine should be avoided as they may cause adverse vasoconstrictor effect. We still lack information about any side effects of alpha-2 agonists in the doses that are recommended in the LC.

Angiotensin II blockade is used as an antihypertensive drug in intensive care and can be an effective complement to beta-1 blockade and alpha-2 agonists in head-injured patients if arterial blood pressure is still too high. It reduces blood pressure with only a limited effect on the cerebral circulation (63, 64). It may also be beneficial by counteracting angiotensin II-induced inflammation (65) and also by reducing vascular permeability, as shown for glomerular permeability in the rat (66). However, there are no studies specifically analyzing the effect of this drug on patients with s-TBI.

No doubt, a markedly raised ICP (above 20–25 mmHg) is a severe negative sign. A raised ICP due to brain edema is more unfavorable than when ICP is increased due to temporary cerebral vasodilation.

PRINCIPLES FOR PERFUSION OF THE PENUMBRA ZONE

Brain cells are the most sensitive cells to hypoxia in the body. A s-TBI patient suffers from various degrees of compromised circulation in the brain. In most cases, there will be one or more severely hypoxic contusion areas, most of the cells of which will not survive independent of therapy. The area just outside the contusions, normally called the penumbra zone, is hypoxic but not dead—and has the potential to survive, especially in its outer (borderline) areas. The risk of severe hypoxia and cell death in less injured parts of the brain outside the penumbra zone is surely smaller. It is reasonable to believe that the degree of impairment of oxygenation of the penumbra zone is essential for outcome. Measures to improve oxygenation of the most injured parts of the penumbra zone would, therefore, be expected to improve outcome.

Oxygenation of the penumbra zone in the injured brain is dependent on the hemoglobin concentration and blood flow

to this area. The flow of blood through a vessel is controlled by its vascular resistance and by the perfusion pressure. A high perfusion pressure accomplished by increase in arterial pressure with vasoconstrictor therapy has been the predominant measure in alternative guidelines, with the specific purpose of ensuring adequate perfusion of the injured brain (4, 5, 46). No doubt perfusion of parts of the brain outside the most injured parts of the penumbra zone is dependent on the perfusion pressure. As explained below, this may, however, not always be the case in the most injured parts of the penumbra zone.

The relation between vascular resistance and vessel radius is formulated by the law of Hagen–Poiseuille (resistance = constant/radius^4). This very unlinear fourth power relation means that the constrictor effect of a vasoconstrictor stimulus is dependent on the initial radius, as shown in **Figure 5**.

From a normal radius (in less injured parts of the brain), there will be just a moderate decrease in flow when influenced by vasoconstrictors, and the flow may even increase if the blood pressure is increased at the same time. In contrast, a vasoconstrictor stimulus in the most injured parts of the penumbra zone—with the radius of the vessels already reduced by the trauma—may result in a marked reduction in blood flow, even though there is a simultaneous increase in arterial blood pressure.

The penumbra zone most likely lacks myogenic response (and autoregulation), but it can still respond to alpha-1 stimulation from release of catecholamines through hypovolemia-induced baroreceptor reflex activation or infused catecholamines (67). As mentioned in the Section "Blood Volume" below, vasoconstrictor therapy also has adverse effects by reducing plasma volume, which, if not compensated for, will cause further activation of the baro receptor reflex and further release of catecholamines. There are indications that high catecholamine levels in TBI patients (endogenously or after noradrenalin infusion) lead to worse outcome in s-TBI patients (59).

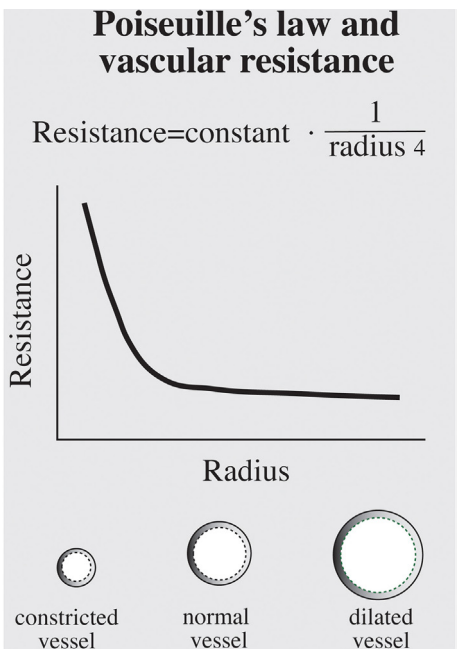

FIGURE 5 | The relation between the flow resistance (*R*) and radius (*r*) of a vessel according to the law of Hagen–Poiseulle. As seen, this is a very unlinear relation (R = constant × $1/r^4$), which means that the effect of a vasoconstrictor is dependent on the initial vascular resistance. The vasoconstriction induced by a vasoconstrictor will be larger in the penumbra zone with a trauma-induced initial vasoconstriction than in less injured areas.

Vasopressors such as noradrenalin, and also phenylephrin and vasopressin, are hypertensive drugs that are still used in alternative guidelines to increase blood pressure in TBI patients. For example, in the SAFE-TBI randomized study in Australia, high doses of noradrenaline were given to maintain CPP above 70 mmHg (68).

As discussed above, the use of vasopressors may reduce circulation, in the most injured parts of the penumbra zone (69), but to some extent also in less injured parts of the brain. Brassard et al. (70) concluded that there is a great risk that infusion of noradrenalin at 0.1 µg/kg/min or higher would negatively affect cerebral oxygenation. As suggested above, there is also a great risk that, through its CPP-increasing effect, noradrenalin increases brain edema. Finally, noradrenaline is a proinflammatory substance with permeability-increasing properties, and it may trigger the development of ARDS (49, 50, 71).

The need for vasopressors is significantly reduced when one accepts the moderate CPP values used in the LC, providing a simultaneous optimal fluid therapy as described under Section "Blood Volume" below. Vasopressors should be avoided, but may be necessary to maintain blood pressure at an adequate level in selected patients with heart failure, multiple injuries, or systemic inflammatory response syndrome (SIRS).

The risk of compromised circulation when treating the patient with antihypertensive drugs according to the LC is small, provided that there is an adequate blood volume substitution therapy and avoidance of vasopressors. This statement found support in a clinical microdialysis study with the microdialysis catheter placed in the penumbra zone. This study showed that TBI patients treated according to the LC could accept a CPP down to 50 mmHg without worsening of the hypoxia (72). Another clinical microdialysis study on patients with s-TBI and raised ICP, who were treated according to the LC, showed a gradual trend of normalization in the penumbra zone of the lactate/pyruvate ratio and glycerol concentration from raised levels. These microdialysis data indicate improved oxygenation and less cell derangement (73), which occurred in spite of the reduced CPP initiated by the antihypertensive treatment in the LC. The improved oxygenation and less cell derangement of the penumbra zone in these studies were most likely an effect of avoidance of hypovolemia, avoidance of vasoconstrictors and of reduced adrenergic stress.

In an attempt to improve the microcirculation of the penumbra zone pharmacologically, a low dose of prostacyclin has been an option in the LC since 1997, with a recommended dose of 0.7–1.2 ng/kg/min (34). Prostacyclin is an endogenous substance released from endothelial cells of the vacular wall. It is a potent inhibitor of platelet and leukocyte aggregation and inhibits their adhesion to the vascular wall and may thereby improve microcirculation. This hypothesis found support from a study in mice

showing that prostacyclin reduces the contusion volume after brain trauma in a dose similar to that recommended in humans (74). Another study on the rat showed improved cortical perfusion with prostacyclin following brain trauma (75). Prostacyclin may increase the risk of bleeding, but only in higher doses than those recommended here. Prostacyclin may also reduce an increased vascular permeability (76).

Two clinical microdialysis studies in s-TBI patients have shown improved oxygenation of the penumbra zone by prostacyclin (77, 78). That prostacyclin improves perfusion of hypoxic areas in a s-TBI patient has also found support from CT scans. **Figure 6** illustrates such an example from an s-TBI patient showing a CT scan just before and another CT scan 4 days after the start of prostacyclin. Maybe it is not a coincidence that one of the best outcome results published from s-TBI patients included treatment with prostacyclin (10). Apparently, prostacyclin may be beneficial in s-TBI patients, but more clinical studies are needed for better evaluation of its composed effect in these patients.

These two prostacyclin studies (77, 78) and the two microdialysis studies presented above (72, 73) illustrate the value of the microdialysis technique in head injury research.

BLOOD VOLUME

The traumatized brain suffers from an initial primary injury followed by a secondary injury. The secondary injury includes an inflammatory response with release of a host of cytokines and a profound acute-phase response, which is thought to contribute to disruption of the BBB and membrane damage of brain cells (19, 20, 79–82). Release of inflammatory substances from the injured brain may also cause SIRS with a general increase in systemic transcapillary leakage of fluid and proteins, and development of hypovolemia (83). This may explain why hemodynamic instability responsive to fluid resuscitation is common shortly after a traumatic brain injury, and also in the absence of systemic hemorrhage (84, 85).

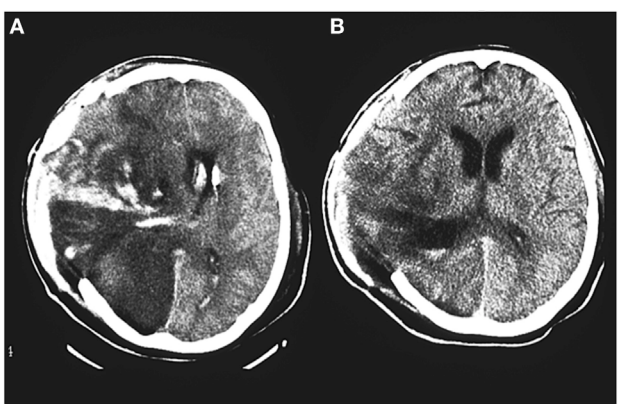

FIGURE 6 | A CT scan of the brain from a 65-year-old woman treated with the Lund concept after a severe traumatic brain injury and craniectomy. **(A)** A CT scan before start of prostacyclin infusion. **(B)** A CT scan after 4 days of prostacyclin infusion (1 ng/kg/min i.v.). There are signs of improved perfusion of an area with compromised perfusion on the right side of the brain.

An s-TBI, also without extracranial bleedings, results in a profound and fast decrease in plasma volume, as shown in a recent study on the cat. In this study, cats were exposed to a standardized fluid percussion brain trauma. The brain trauma resulted in 15% reduction in plasma volume, as measured 3 h after the trauma (86). In spite of the reduction in plasma volume, there was an increase in arterial blood pressure, most likely due to a trauma-induced hyperadrenergic state, showing that blood pressure is an unreliable parameter for evaluation of hypovolemia after a brain trauma.

In a study by Rise et al. (87) on anesthetized pigs, it was found that just a moderate hypovolemia, which had no adverse effects in normal pigs, resulted in compromised brain circulation after a brain injury. These authors suggested that alpha stimulation *via* activation of the baroreceptor reflex contributed to the compromised circulation (67, 87). A mechanism based on the law of Hagen–Poiseulle, as described under the section "Principles for Perfusion of the Penumbra Zone" above, may explain why the sensitivity to adrenergic vasoconstriction in the penumbra zone is increased after a brain trauma (**Figure 5**). This supports the hypothesis adopted in the LC that avoidance of hypovolemia is essential for good outcome after a brain injury.

HOW TO PREVENT HYPOVOLEMIA

If the patient suffers an extracranial hemorrhage in combination with the head injury, the bleeding must be stopped and possible replacement of the blood loss by erythrocyte and plasma volume transfusion must be considered. If there is no extracranial hemorrhage, hypvolemia will still develop by transcapillary leakage. The degree of hypovolemia can be reduced by infusion of plasma volume expanders, and also by measures that counteract transcapillary leakage. Possible physiological mechanisms that counteract transcapillary leakage and physiological considerations regarding the effectiveness of various plasma volume expanders will be discussed below.

Even though there are difficulties in estimating the exact degree of hypovolemia, it can be roughly estimated with standard methods— such as by analyzing the arterial pulse pressure curve configuration, observing the blood pressure response upon a bolus infusion, or leg elevation. Note that the probability of hypovolemia is greater with low hematocrit than with normal hematocrit (see "Erythrocyte Transfusion" below). Most s-TBI patients will develop hypovolemia if not properly treated.

Nowadays, the 2-pore model is a generally accepted model to explain transcapillary fluid exchange. It means that the capillary membranes outside the brain consist of a large number of small pores along the whole capillary membrane and only permeable for small solutes, and less frequent larger pores also permeable for larger molecules such as proteins, at the end of the capillary network and in venules (88). A schematic illustration of the 2-pore model is shown in **Figure 7**. All the pores are situated between the endothelial cells.

The two Starling forces, the hydrostatic and the oncotic transcapillary pressures, control fluid exchange through the small pores. The mechanisms that control fluid flow across the large pores are somewhat different. Due to the free permeability of

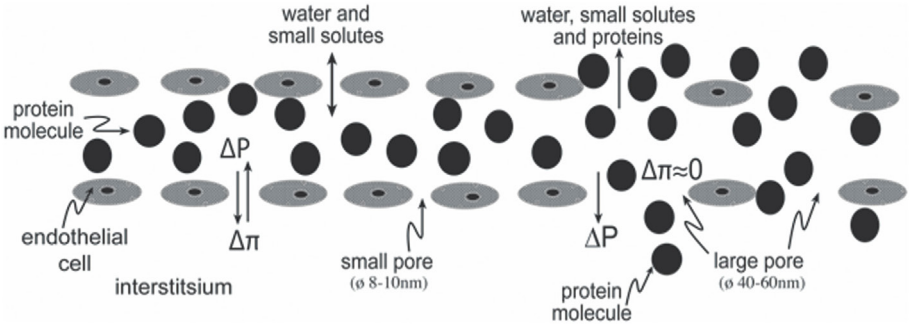

FIGURE 7 | Transcapillary fluid exchange across a capillary outside the brain, according to the 2-pore theory. The illustration shows the large number of small pores across the whole capillary membrane that are permeable only to water and small molecules, such as electrolytes, and the much fewer large pores at the end of the capillary network and in venules, which are also freely permeable to proteins. No erythrocytes are shown. Due to the same oncotic pressure on both sides of the large pores, there is no oncotic absorbing force across the large pore ($\Delta\pi = 0$). This means that the transcapillary hydrostatic pressure (ΔPc) is the only force across the large pores, creating a convective protein-rich volume flow through them. This passive transcapillary volume flow will increase after an increase in arterial pressure, increasing the risk of protein loss and hypovolemia. There will be an increase in the number of large pores after trauma, which will increase the transcapillary loss of proteins. This theory means that protein loss is a passive mechanism, mainly through convection. For details, see Ref. (88). Partly reproduced from Ref. (8) with permission.

proteins through the large pores, there will be about the same oncotic pressure on the intravascular and interstitial side of the pore. This means that the hydrostatic pressure is the predominant pressure force in the large pores, creating a non-energy-dependent passive filtration. Proteins will follow the fluid stream to the interstitium through the large pores, mainly by convection (88), and transcapillary loss of proteins occurs passively without energy-dependent active trancytosis.

The 2-pore model means that an increase in hydrostatic capillary pressure will increase transcapillary loss of fluid and electrolytes through both small and large pores, while transcapillary loss of proteins mainly occurs *via* convection through the large pores. This hypothesis was confirmed experimentally in a study on rats with SIRS, showing a significant loss in plasma volume following an increase in arterial blood pressure through noradrenaline infusion (**Figure 8**) (89). In this study, a plasma volume loss of about 3 mL/kg was measured 2.5 h after an albumin transfusion of 15 mL/kg in the control group with baseline arterial pressure, as compared to a corresponding loss of about 14 mL/kg when the mean arterial pressure was increased by about 12 mmHg by noradrenalin infusion. That there is an increase in plasma volume loss following noradrenalin infusion has also found support in a clinical study on patients with a large thoracic surgical trauma (90). Hydrostatic capillary pressure increases after noradrenalin infusion not only because of an increase in arterial pressure but also because of postcapillary venular vasoconstriction. The permeability-increasing effect of noradrenalin may also contribute to the plasma fluid loss.

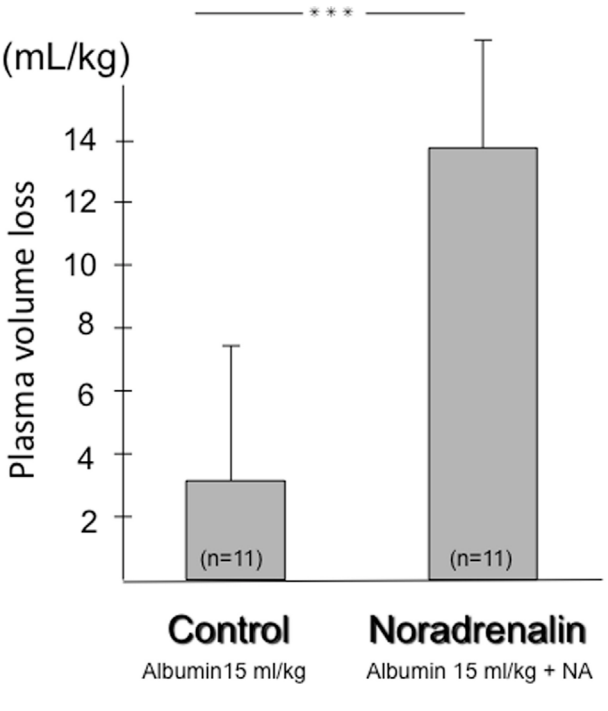

FIGURE 8 | Effects on plasma volume in the septic rat of a bolus infusion of 5% albumin of 15 mL/kg, measured 2.5 h after the infusion in a control group with baseline mean arterial blood pressure and in a group where mean arterial blood pressure was increased by 12 mmHg using noradrenalin infusion. The loss of plasma volume was significantly greater in the noradrenalin group (14 mL/kg as opposed to 3 mL/kg), with loss of the main part of the infused volume. Data from Ref. (89), with permission.

FLUID SUBSTITUTION

Optimal fluid substitution is important for s-TBI patients, to prevent hypovolemia. As mentioned above, there is a risk that s-TBI patients develop hypovolemia very soon after the trauma, even if there is no extracranial bleeding (86). Isotonic crystalloids (e.g., saline or ringer lactate) and albumin are currently the only plasma volume expanders that can be recommended for s-TBI patients. At low hemoglobin concentration, blood erythrocyte transfusion will also increase the blood volume (see Erythrocyte Transfusion below).

Crystalloids are poor plasma volume expanders, as only 20–30% of the infused volume or even less will stay intravascularly. The rest (70–80%) will reach the interstitial space within 20 min after the infusion, contributing to general systemic tissue edema (91, 92). If only a crystalloid solution is used, there is a need for large fluid volumes to prevent hypovolemia.

There may also be a significant passive distribution of crystalloid fluids to the interstitium of the injured brain with a disrupted BBB. The use of crystalloids as plasma volume expander, therefore, may increase brain edema and ICP, especially if hypotonic solutions are used (93, 94). Still, infusion of moderate volumes of crystalloid solutions is important to maintain the fluid balance with normal urine production. Saline is the most common crystalloid used for s-TBI patients, but ringer lactate is an alternative. In larger volumes saline may lead to adverse hyperchloremic metabolic acidosis. A more balanced crystalloid solution may be a good choice.

Albumin, in combination with saline, has long been a standard plasma fluid expander in TBI patients. Apart from the absorbing effect of the increase in plasma oncotic pressure, which might reduce brain edema (95), albumin has a plasma-expanding effect, preventing hypovolemia, and thereby improving microcirculation in the penumbra zone. The plasma volume effect of albumin will, however, be reduced by the increase in transcapillary leakage of proteins, which may occur after s-TBI. The rate of this leakage, called the transcapillary escape rate, is normally 5–6% of the total plasma volume per hour, but can at least double after trauma and during systemic inflammation. The leaked albumin is transferred back to the circulation *via* the lymphatic system. When the leakage is greater than the lymphatic recirculating capacity—due to increased protein leakage or reduced lymphatic capacity—there will be a reduced protein concentration in plasma and interstitial accumulation of proteins. Simultaneously with their use as thrombosis prophylaxis, active physiotherapy and intermittent pneumatic leg compression may increase the recirculating capacity of the lymphatic system in arms and legs.

A retrospective outcome study on s-TBI patients showed that the concentration of albumin in plasma decreased considerably from normal values in the first days after trauma, and that low albumin levels were predictors of bad outcome (96). The authors suggested that low albumin levels are potential for albumin treatment. There have been several smaller single-center studies giving support for the use of albumin in s-TBI patients (97, 98). A study in rats showed that the use of albumin resulted in less brain edema after a head trauma than the use of saline in corresponding plasma-expanding doses (99). Albumin has been found to improve organ function in critically ill hypoalbuminemic patients (100). Another study in rats showed that albumin infusion improved systemic microcirculation and global hemodynamics and attenuated the inflammatory response to reperfusion by reducing rolling of leukocytes (101).

Only one study, the randomized SAFE-TBI study in Australia and New Zeeland, has found a worse outcome in s-TBI patients with albumin than with saline (68). The worse outcome with 4% abumin than with saline in that study was suggested to be an effect of raised ICP, without giving any more specific explanations (102). The SAFE-TBI study, however, has been called into question (103–106). It was a subgroup analysis involving 321 patients selected from an intensive care material of 7,000 patients. Subgroup analysis can be criticized because of the risk that the two groups may differ at baseline (107), as was also to the case in this study regarding baseline ICP and the number of patients with age above 55 years, both differences favoring the saline group. What is more important when criticizing this study is the use of hypotonic albumin solution of 255–260 mosm/L (whereas normal plasma osmolality is 290–300 mosm/L). It has been well established that hypotonic solutions are contraindicated in s-TBI patients, due to the risk of development of brain edema (93, 94). Ertmer and Van Aken (105) declared that the colloid compound was not the deleterious factor in the SAFE-TBI study, but that the study just confirmed that hypotonic solutions are deleterious in TBI patients. It was concluded by Ioannidis that evidence from trials, no matter how impressive, should be interpreted with caution when only one trial is available (108), a statement applicable for the SAFE-TBI study.

Considering the critic raised and the unexpected results of the SAFE-TBI study, it is a reasonable assumption that this study alone cannot be used to question the use of albumin in s-TBI-patients.

Severe traumatic brain injury patients require an effective plasma volume expander to avoid hypovolemia, but currently there is no such optimal plasma volume expander available. For the reasons described above, the use of a crystalloid as the only plasma volume expander to maintain normovolemia must be seen as a theoretical drawback for TBI patients for reasons described above. A crystalloid such as saline in combination with albumin should be a more reasonable choice. So far, there are no reasons to change the recommendations in the LC of a mixture of isotonic albumin (preferably 20% albumin) and saline as plasma volume expanders to s-TBI patients (3, 8, 9). It should be a reasonable goal to maintain albumin concentration of at least 32 g/L.

ERYTHROCYTE TRANSFUSION

Erythrocytes contribute to a large proportion (normally 40%) of the intravascular volume. This means that there is a larger intravascular volume to be replaced by plasma volume expanders, to maintain normovolemia, at a low hemoglobin concentration than at a higher hemoglobin concentration. Erythrocytes may also reduce plasma volume leakage, as has been shown in studies on dogs and rats (109, 110). A hemoglobin concentration far below normal may even mean difficulties to maintain normovolemia.

Several studies have shown improved oxygenation of the brain after red blood cell transfusion (111–114). Considering the hypoxic penumbra zone, patients with s-TBI may represent a population of individuals who are particularly susceptible to anemia and hypovolemia. It is, therefore, reasonable to believe that s-TBI patients cannot be compared with general intensive care patients regarding possible beneficial effects of blood transfusion. There are theoretical arguments for giving blood transfusions specifically to s-TBI patients at a low hemoglobin concentration, with the purpose of improving oxygenation of

the penumbra zone, to reduce cytotoxic brain edema and to maintain normovolemia. That's why erythrocyte transfusions of up to a hemoglobin concentration of above 110 g/L has been recommended previously to s-TBI patients in the LC, and always with leukocyte-depleted blood (3, 8). As described below some adjustment of recommended hemoglobin level toward somewhat lower levels has been done.

A study by McIntyre et al. showed a not fully significantly improved outcome in s-TBI patients with a liberal transfusion strategy (a 60-day mortality of 17 vs 13%) (115), and these results even though the blood was not leukocyte-reduced in this study. The literature shows varying results regarding the effects on outcome after blood transfusion in s-TBI patients, as summarized in a recent review (116). Despite the potentially improved oxygenation of the penumbra zone and less risk of hypovolemia, some studies have indicated worse outcome after blood transfusion, whereas others have not (117, 118). The quality of the blood may be a factor of great importance. Perhaps the most negative characteristic of transfused red blood cells is the leukocyte content. For example, leukocytes have been recognized to be a strong contributor to a number of adverse effects of blood transfusion. Leukocytes are proinflammatory, and outcome in the intensive care unit is improved and fever reduced when using leukocyte-depleted blood instead of non-leukocyte-depleted blood (119–122). Also, the storage time is of importance for the quality of blood (123). In most of the erythrocyte transfusion studies presented so far on s-TBI patients, non-leukocyte-depleted blood has been used. Interpretation of the results may also be difficult when the low hemoglobin concentration and the use of blood transfusion reflect the degree of illness (117), and the amount of blood transfused reflects severity of injury. For example, in the study by Salim et al. (117), the patients given blood were older, were more severely injured from the start, and had a lower rating on the Glascow Coma Scale.

The current knowledge regarding the effect on outcome of erythrocyte transfusion in patients with TBI is highly conflicting. We still lack any strong indications that erythrocyte transfusion is beneficial for outcome in s-TBI patients, in spite of the fact that many studies have shown improved oxygenation and that anemia with hemoglobin below 9 g/L is a predictor of bad outcome (26), and that blood is a good blood volume expander. The improved oxygenation and our non-scientific experience of a hemodynamically more stable patient with a tendency of reduced ICP after blood transfusion cannot be enough for general acceptance of erythrocyte transfusion. We can never overcome the fact that blood transfusions are transplantations from other individuals, with many still unknown adverse components. That leukocyte-depleted blood was not used in most studies may have been of importance for the outcome in these studies. There has been no randomized study of the optimal hemoglobin concentration in neurocritical care. Due to the lack of convincing scientific support for our original hypothesis in the LC of improved outcome with a relatively liberal use of transfusion with leukocyte-depleted blood, we can recommend a somewhat more restrictive use of blood to s-TBI patients. A new target hemoglobin level could be 105–110 g/L, always with leukocyte-depleted blood.

PLASMA-SPARING COMPONENTS OF THE LC

Some of the components of the LC (denoted I–VI below) may help to reduce the transcapillary leakage of plasma fluid and plasma proteins, and thereby the need for plasma volume expanders and counteracting hypovolemia.

The 2-pore model (**Figure 7**) means that transcapillary loss of plasma fluid and plasma proteins is highly dependent on the hydrostatic capillary pressure, as also confirmed both experimentally (89) (**Figure 8**) and clinically (90). If so, the use of antihypertensive therapy (I) and avoidance of vasopressors (II) will reduce the need for plasma volume expanders. As discussed above, the use of albumin (e.g., 20% isotonic solutions) (III) and avoidance of hemoglobin concentrations that are too low (IV) may help to counteract transcapillary loss of fluid.

A study in septic guinea pigs showed that a slow rate of infusion of albumin has better plasma-expanding effect than a fast infusion rate (124). If so, a low rate of infusion of albumin may improve its plasma-expanding effect (V).

Frequent physiotherapy and intermittent pneumatic leg compression with antithrombotic stocks may not only counteract deep leg thrombosis but may also help to bring back interstitial fluid to the circulation by increasing the capacity of the lymphatic recirculating system in the legs (VI). Components I–VI have been presented in **Table 2**.

The effectiveness to spare plasma for each of these six components is not evaluated, but our experience supports that these measures taken together reduce the need for albumin.

VENTILATION

All patients with an s-TBI treated with the LC are mechanically ventilated. Volume-controlled ventilation is preferred to minimize variation in arterial P_{CO_2} during secrete stagnation. In line with the general goal in the LC of maintaining normality as far as possible, the TBI patients are normoventilated, keeping arterial P_{CO_2} within a normal range (4.5–5.0 kPa). Underventilation should be avoided because of the risk of cerebral hyperemia with increase in intracranial blood volume and ICP. Hyperventilation, on the other hand, means a risk of vasoconstriction and increased hypoxia, especially in the penumbra zone and is not recommended (125, 126). Hyperventilation down to arterial P_{CO_2} of 25 mmHg for the purpose of reducing ICP is still accepted in some alternative guidelines (e.g., the Brain Trauma Foundation guideline from 2016) (7).

TABLE 2 | Potential measures adopted in the Lund concept to reduce transcapillary leak and the need of albumin (and crystalloids) as plasma volume expander.

(I) Avoidance of high arterial pressure with antihypertensive drugs
(II) Avoidance of vasopressors
(III) The use of albumin as plasma volume expanders, preferably 20% solutions
(IV) Avoidance of low hemoglobin concentrations
(V) Low infusion rates of albumin
(VI) Physiotherapy and antithrombotic pneumatic leg compressions

Oxygen concentration in the ventilator should be set to give a normal arterial P_{O_2} of around 12 kPa, helping to optimize oxygenation of the penumbra zone. Higher arterial P_{O_2} should be avoided because of risk of hyperoxic cerebral vasoconstriction and hyperoxic lung damage (127, 128).

High-dose barbiturate and noradrenalin treatment may trigger pulmonary insufficiency and fever, as mentioned under Section "Sedation" below and under section "Principles for Perfusion of the Penumbra Zone" above (50) and was not used in the LC. PEEP is obligatory to prevent atelectasis and is safe for the brain, as discussed above under Section "Hemodynamic Consequences of the Rigid Cranium." Tracheostomy can be considered in selected cases.

Intermittent cautious moderate bagging under ICP control and inhalation to prevent secrete stagnation and atelectasis have been recommended in the LC. Note that inhalation with beta-2 stimulating drugs may induce transient vasodilation with increase in ICP and decrease in blood pressure—and, if so, the dose should be reduced to half the dose or less when starting inhalation next time.

ACTIVE COOLING

The concept of active cooling of s-TBI patients was discussed in detail in a recent review (129). Active cooling of patients with TBI was first described by Fay in 1945 (130) and has become a major area of research during the last 2–3 decades. Based on the convincing neuroprotective effect of hypothermia under hypoxia as illustrated by case reports showing good recovery after drowning in cold water (129), great expectations have been raised about active cooling as a breakthrough in s-TBI patients (131). In spite of this, general active cooling has never become a component of the LC, mainly because of the pathophysiological arguments presented below.

Even though cooling is neuroprotective and may improve outcome after a general brain hypoxia, the situation is different after s-TBI. The traumatized brain often suffers from compromised circulation and hypoxia in and around its most injured areas, the penumbra zone. It may, therefore, be extra sensitive to the hyperadrenergic stress induced not only by the brain trauma itself (42–44) but also by that induced by active hypothermia. Active cooling means that the temperature of the body becomes lower than the value set in the biological thermostat. This difference creates a marked adrenergic stress superimposed on the head trauma-induced stress, with shivering, increased sympathetic discharge, and catecholamine release. The hypothermia-induced stress may further compromise circulation of the penumbra zone. The powerful hypothermia-induced adrenergic stress is aimed at resetting the body temperature to the value set in the biological thermostat of the brain. This view has found support from studies showing a significant reduction in brain oxygenation following active cooling simultaneously with an increased metabolism of the most injured parts of the brain (132–134).

There are other complications attributed to hypothermia, such as coagulopathy, cardiovascular complications, and especially, pneumonia (129, 135). A Cochrane analysis pointed out the risk of pulmonary complications with active cooling (136).

These pathophysiological principles and consequences may explain why none of the randomized high-quality hypothermia studies performed during the last —two to three decades in s-TBI patients have shown any beneficial effects on outcome; some have even indicated that active cooling is detrimental for outcome. The randomized hypothermia studies in s-TBI patients performed are presented below.

The well-designed pediatric studies by Hutchison et al. (137) and by Adelson et al. (138) showed no significant differences in outcome between the hypothermia groups and the corresponding control groups. In the Hutchinson study, there was a significantly higher mortality in the hypothermia group in a subgroup of patients over 7 years of age—with a mortality rate of 21% as compared to 12% in the control group.

The two studies by Clifton et al. (139, 140) can also be classified as high-quality studies. There was no significant difference in mortality between the hypothermia group and the normothermia group in these studies, but there was a tendency of worse outcome in the hypothermia group with a longer hospital stay and more complications.

That hypothermia lowers a raised ICP is well known from most hypothermia studies [e.g., Ref. (131)]. This is most likely an effect of hypothermia-induced vasoconstriction, with a simultaneous reduction in cerebral blood flow and blood volume. This experience initiated a large multicentre European study evaluating the effect on outcome of hypothermia to 33–35°C in patients with an ICP above 20 mmHg (134). In this well-performed study, as expected, they found that ICP decreased with hypothermia, but there was no improvement in outcome. If anything there was a tendency of worse outcome in the hypothermia group, and the study was interrupted prematurely. Later analysis of the material from this study also showed that hypothermia reduced oxygenation of the brain.

To summarize, the best hypothermia studies taken together from the last two decades have shown that hypothermia does not improve outcome or, if anything, they have shown a tendency of worse outcome in s-TBI patients. This conclusion finds support from a Cochrane study, which concluded that head trauma patients treated with hypothermia, were slightly more prone to die (136). Active hypothermia is no longer recommended in the Brain Trauma Foundation guidelines from 2016. Therefore, the recommendation made in the LC 25 years ago that TBI patients should not be treated with active hypothermia (3, 8) has found strong support during the last two decades.

FEVER

It is believed that high fever is deleterious in TBI patients (141). It appeared that high fever in s-TBI patients was much less common in our intensive care unit after introduction of the LC, which may have several explanations. Pulmonary complications in terms of atelectasis and pneumonia were reduced by PEEP, by avoidance of vasopressors and especially by avoidance of high-dose treatment with barbiturate. Change from high calories parenterally to lower calories, mainly with enteral nutrition, may also counteract

the development of fever (see "Nutrition" below). Paracetamol reduces fever by affecting the biological thermostat. The effect, however, is relatively small (temperature reduction by 0.5°), but is used in the LC.

Steroids might be an alternative to reduce fever by affecting the biological thermostat. Use of steroids to TBI patients, however, can be questioned after the CRASH study, which showed worse outcome in TBI patients (most of them without fever) treated with high doses of metylprednisolone (in total 22 g for 2 days) (142). As mentioned above, active general cooling is not a therapeutic component of s-TBI patients in the LC, but if temperature is persistently high (>39.5–40°C), it must be reduced in some way—and in this situation, a more long-term active cooling or a bolus dose of a steroid (e.g., metylprednisolon 0.25–0.5 g) may be life-saving.

OSMOTHERAPY

Osmotherapy with mannitol has been used since the beginning of the 1960s as the main treatment for a raised ICP and is still a main component of most s-TBI guidelines. Hypertonic saline has become an alternative osmotic drug during the last 20 years. In spite of its use for more than 55 years, and more than 155 publications, the scientific world has failed to come up with reliable beneficial data on outcome related to osmotherapy.

A Cochrane analysis did not find any beneficial effects on outcome of osmotherapy (143), a conclusion that was in agreement with that in a recent review on osmotherapy (144). The only studies showing favorable effects of mannitol on outcome appeared to be faked (145). Osmotherapy effectively reduces ICP, but mannitol especially has severe side effects in terms of renal and pulmonary failure, electrolyte disturbances, and a rebound increase in ICP after the mannitol infusion is terminated (143).

The rebound increase in ICP after the mannitol infusion is discontinued may be an effect of intracellular accumulation of mannitol in the brain creating an osmotic fluid filtration force when plasma mannitol concentration is reduced after the infusion is stopped. A rebound effect of mannitol has also been demonstrated experimentally on cat skeletal muscle enclosed in a plethysmograph (146). It can be speculated that the rebound effect could to some extent also be an effect of increased transcapillary pressure following the mannitol-induced reduction in ICP (see Decompressive Craniectomy below).

In combination with its side effects, mannitol treatment has the weakness of just treating the symptom (raised ICP) without attacking the pathophysiological mechanisms behind the symptom. There are apparently arguments against the use of osmotherapy, and especially mannitol. Except from acute prevention of brain stem compression, osmotherapy has not been a component of the LC. During the 25 years that LC has existed, no new information has been presented to support any change in the recommendation in the LC regarding osmotherapy (3). The need of osmotherapy in LC is reduced by other ICP-reducing measures, such as antihypertensive and sedative therapies.

DECOMPRESSIVE CRANIECTOMY

After some sceptisism during the 90th decompressive craniectomy has nowadays become included in most TBI guidelines. Early on, it became a component of the LC in patients with uncontrolled high ICP after other measures to reduce ICP had failed. Craniectomy reduces ICP by giving extra space to the swollen brain, and it may quickly prevent brain stem compression and death. Side effects with craniectomy may be reduced by considering its hemodynamic consequences, as clarified below with the help of **Figure 2**.

A reduction in ICP after decompressive craniectomy means an increase in transcapillary hydrostatic pressure. This will increase the transcapillary filtration, creating more brain edema and expansion of the swollen brain, especially in the cranial opening—as routinely seen on the CT scan. If not counteracted, the development of brain edema is a potential drawback of craniectomy with the risk of herniation, axonal stretch, and strangulation in the cranial opening (147, 148). As discussed below, brain edema after craniectomy can be reduced when considering hemodynamic aspects for development of vasogenic brain edema.

After craniectomy, there is often a sudden marked reduction in the raised arterial pressure simultaneously with a drastic reduction in ICP. This is often followed by a moderate increase in ICP (149). It can be speculated that this increase in ICP after cranietomy is an effect of the unbalance in the Starling fluid equilibrium following the initial lowering in ICP. The spontaneous reduction in arterial pressure in combination with an active treatment with antihypertensive drugs and treatment with albumin should counteract the development of brain edema in the cranial opening.

According to these theories, CPP should be kept at a relatively low level with antihypertensive treatment after craniectomy. This may explain the beneficial outcome after craniectomy in a study in which the LC therapy was used (149). In that study, there was no difference in outcome between non-craniectomized patients and craniectomized patients, even though the craniectomized patients had higher ICP initially.

These hemodynamic principles may give a hint as to why outcome results after craniectomy differ between studies. In a randomized study in Australia by Cooper et al. (150), unfavorable outcome was found after craniectomy, while a randomized study in Cambridge showed improvement in outcome after cranietomy, but at the expense of more disability (151). The study by Cooper et al. has been criticized for the low entry criterion for ICP of 20 mmHg and that the study gave no information about CPP and the use of vasopressors (150). It is well known that vasopressors are frequently used in TBI patients in Australia (e.g., the SAFE-TBI study) keeping CPP above 70 mmHg (68).

It seems that decompressive craniectomy may be a life-saving measure for prevention of brain stem herniation and death at an uncontrolled high ICP, if otherwise optimal treatment is used. Decompressive craniectomy is a component of the LC.

OTHER SURGICAL MEASURES

Evacuation of hematomas and focal lesions and CSF drainage, also mean loss of transcapillary counter-pressure with the

risk of a subsequent brain edema development, providing a disrupted BBB.

Removal of space occupying hematomas and superficial contusions will reduce a raised ICP and may improve outcome, and is accepted in the LC. The improved outcome most likely is not only an effect of a decrease in intracranial volume but also an effect of reduced release of toxic substances from hematomas and contusions (3).

Ventricular drainage of CSF can sometimes be an effective way of controlling ICP. There is always a risk, however, that the volume of drained CSF is replaced by edema when transcapillary pressure is increased after reduction of ICP by the drainage procedure—with increased risk of ventricular collapse. Such a scenario is shown in **Figure 9**. CSF drainage was started at an ICP of 32 mmHg at a drainage level of about 22 mmHg (**Figure 9A**). After 1 day of drainage, there was a smaller ventricular volume (**Figure 9B**). The drainage was now closed and the ventricular volume was partly recovered about 2 days thereafter (**Figure 9C**). **Figure 9** gives support for principles for transcapillary exchange in the injured brain as described under Section "Principles for Brain Volume Regulation" above.

Reduced ventricular volume during drainage can be prevented if intermittent drainage is used or if the drainage level is set at a level that is not too far below the initial ICP level (e.g., 2–4 mmHg), followed by a successive slow reduction over days. Ventricular sizes should be followed regularly with CT scan to discover incipient ventricular collapse. Ventricular drainage is also recommended in the last Brain Trauma Foundation guidelines (7), but the drainage level is not specified. The ventricular collapse effect following CSF drainage may explain why we still lack studies showing a beneficial outcome effect of ventricular drainage.

NUTRITION

At the time of introduction of the LC 25 years ago, there was a general recommendation that adult patients in intensive care should be given high-energy nutrition of up to 3,000 kcal every 24 h to counteract catabolism. To achieve this energy supply, large volumes of parenteral nutrition with lipids in combination with amino acids and glucose were used. Our metabolic measurements in adult s-TBI patients, however, showed a basal metabolism in these sedated, adrenocortically depressed, and artificially ventilated patients of only 1,200–1,300 kcal/24 h or less.

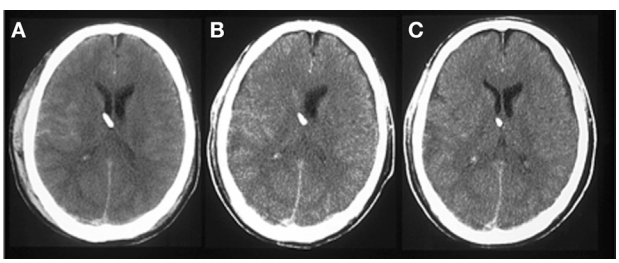

FIGURE 9 | A CT scan of the brain from a 45-year-old man after a severe traumatic brain injury. **(A)** A CT scan before the start of drainage at an intracranial pressure of 32 mmHg. **(B)** A CT scan after 1 day of ventricular drainage, at a drainage level of 22 mmHg. **(C)** CT scan 2 days after drainage was stopped. Ventricular size was reduced by the drainage, and partly restored after the drainage was stopped. The figure supports the presented principles for transvascular fluid exchange in the injured brain with disrupted blood–brain barrier.

At that time, new ideas were being introduced that overnutrition should be avoided, as more energy than the basal need cannot be utilized in highly catabolic patients, creating fever. When we changed from high-energy, mainly parenteral nutrition to a more low energy, mainly enteral nutrition, we noticed less fever in our s-TBI patients. It is well known that parenteral overnutrition with fat can induce hemophagocytosis and fever (152) and that infections are more common in patients who are treated with parenteral nutrition than with enteral nutrition. Since then, our recommendation in the LC has been an initial energy supply from day 2 of 15–20 kcal/kg/24 h to the adult—mainly enteral nutrition, if necessary complemented with infusion of intravenous 5% glucose—later followed by a slow increase in the supply. This regime should be followed to prevent malnutrition and overnutrition. Children need more energy per kilogram. This relatively low-energy supply means that most of the nutrition can be given through the natural and more beneficial enteral form. If necessary to reach enough caloric supply, it can be complemented with glucose solutions with electrolytes. Avoidance of both overnutrition and malnutrition has become a generally accepted goal in the intensive care unit (153). Blood glucose should not be in the lower range and be kept between 6.0 and 8.5 mmol/L, if necessary with insulin, which is in agreement with the NICE SUGAR study (154).

SEDATION

Reducing general stress and the adrenocortical influence is an important component of the LC. Even unconscious TBI patients can be severely stressed, with increased blood pressure and ICP. Extra stress may occur during tracheal suctioning and from alarms and other noises in the room, wake-up tests, and insufficient sedation. The increased adrenocortical stress in TBI patients is reduced in the LC by the use of antihypertensive drugs, such as beta-blocker, alpha-2 agonist, and sometimes angiotensin II agonist and by avoidance of the use of catecholamines (see above). The stress is also reduced in the LC by sedation with, midazolam, and fentanyl—which also makes wake-up tests inexpedient in the LC (3).

Heavy sedation may have pulmonary side effects. Therefore, the degree of sedation should not be deeper than what is necessary to effectively reduce stress, and the seemingly unstressed patient should not be given aggressive sedation. The degree of sedation should be successively reduced in relation to the observed decrease in ICP with a change toward short-acting sedatives, such as propofol before start of the weaning phase. The depth of sedation aims at avoiding normal clinical signs of stress such as inoppropriate movements and coughing combined with an increase in ICP. Due to the anti-stress therapy, epileptic seizures are very rare or absent with the LC, as shown in a separate study (155). Prophylactic anti-convulsive treatment is, therefore,

not a component of the LC. We normally do not measure depth of sedation with BIS or cEEG.

High-dose barbiturate treatment was common in TBI patients in our intensive care unit before we started with the LC in 1991–1992. At that time, we had severe complications in TBI patients in terms of pulmonary complications and frequent ARDS, and quite often very high fever. An experimental study in the cat showed that a high dose of barbiturates, except that it caused complete inhibition of autoregulation of blood flow, it caused an almost complete inhibition of the actin-myosin activity in vascular smooth muscle cells (156). A similar inhibition of spontaneous actin-myosin bronchial ciliae activity may result in a drastic inhibition of the self-cleaning capacity of the lung, with increased risk of ARDS and pneumonia. We cannot tell for sure whether such a mechanism contributed to the high frequency of ARDS, pneumonia, and fever in TBI patients at that time, but these lung problems were drastically reduced after high-dose barbiturate therapy was stopped. Barbiturates are still given in the LC in selected cases, but only as an extra sedation and to restrict a life-threatening high ICP—and only low doses are used (<2–3 mg/kg/h) for 2 days at most (3). No studies have shown improved outcome with barbiturate therapy (157).

Still, high-dose barbiturate treatment in doses below those giving burst suppression pattern is accepted in some alternative guidelines (7). Both barbiturate treatment and mannitol treatment suffer from the weakness that they just treat the symptom of raised ICP, without addressing the pathophysiological mechanisms behind the raised ICP.

SUMMARY

In the present review, I have described some hemodynamic principles, that may be of value in understanding various scenarios in the traumatized brain, and when formulating treatments for s-TBI patients. I have also discussed certain aspects of components such as temperature, ventilation, nutrition, osmotherapy, decompressive craniectomy, and sedation. Most of the principles discussed find support from physiological and pathophysiological principles and from clinical and experimental studies from our and other groups. Clinical confirmation of some components, such as liberal use of blood transfusion with leukocyte-depleted blood, is still lacking. For doses of the different drugs used (3).

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The author confirms being the sole contributor of this work and approved it for publication.

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Anatomical and Physiological Differences between Children and Adults Relevant to Traumatic Brain Injury and the Implications for Clinical Assessment and Care

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General and central nervous system anatomy and physiology in children is different to that of adults and this is relevant to traumatic brain injury (TBI) and spinal cord injury. The controversies and uncertainties in adult neurotrauma are magnified by these differences, the lack of normative data for children, the scarcity of pediatric studies, and inappropriate generalization from adult studies. Cerebral metabolism develops rapidly in the early years, driven by cortical development, synaptogenesis, and rapid myelination, followed by equally dramatic changes in baseline and stimulated cerebral blood flow. Therefore, adult values for cerebral hemodynamics do not apply to children, and children cannot be easily approached as a homogenous group, especially given the marked changes between birth and age 8. Their cranial and spinal anatomy undergoes many changes, from the presence and disappearance of the fontanels, the presence and closure of cranial sutures, the thickness and pliability of the cranium, anatomy of the vertebra, and the maturity of the cervical ligaments and muscles. Moreover, their systemic anatomy changes over time. The head is relatively large in young children, the airway is easily compromised, the chest is poorly protected, the abdominal organs are large. Physiology changes—blood volume is small by comparison, hypothermia develops easily, intracranial pressure (ICP) is lower, and blood pressure normograms are considerably different at different ages, with potentially important implications for cerebral perfusion pressure (CPP) thresholds. Mechanisms and pathologies also differ—diffuse injuries are common in accidental injury, and growing fractures, non-accidental injury and spinal cord injury without radiographic abnormality are unique to the pediatric population. Despite these clear differences and the vulnerability of children, the amount of pediatric-specific data in TBI is surprisingly weak. There are no robust guidelines for even basics aspects of care in children, such as ICP and CPP management. This is particularly alarming given that TBI is a leading cause of death in children. To address this, there is an urgent need for pediatric-specific clinical research. If this goal is to be achieved, any clinician or researcher interested in pediatric neurotrauma must be familiar with its unique pathophysiological characteristics.

Keywords: children, traumatic brain injury, neurotrauma, brain, head

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WHY CHILDREN AND ADULTS ARE DIFFERENT

Adult physicians often underestimate the differences between adults and children. Those who work with children seldom do. Although children *are* very different from adults in physiology and disease, we commonly extrapolate data from adult traumatic brain injury (TBI) studies to pediatrics. At best this is often inappropriate; at worst it may be dangerous. The problem is that there are fewer studies in children, and so less evidence on which to base recommendations. Children are seen as a vulnerable population in ethics terms and so extrapolation from adult data is encouraged, which contributes to this practice. Its unintended consequence is weakened evidence to direct treatment for this most vulnerable population. This may be defendable if children were easier to treat than adults but unfortunately the converse is true. All of the difficulties and controversies of adult TBI are compounded in children. There are many examples. In children, the debate about thresholds for intracranial pressure (ICP) treatment are aggravated by the fact that normative values for ICP in children are not well established and depend on age. The same is true for blood pressure (BP), and so uncertainty about optimal cerebral perfusion pressure (CPP) thresholds is even greater. Resting and activated metabolic rates change across the childhood age range before settling into a reasonably stable pattern in adulthood, as does cerebral blood flow (CBF) and its response to injury. Clinical assessment is challenging—there are differences in the expected patterns of injury, clinical evaluation, imaging, and outcome assessment. Differences abound also in surgery: children have smaller blood volumes, reduced tolerance for blood loss, increased risks of long anesthesia, different reactions to medications, and reduced tissue perfusion—these are all challenging in children and so require special knowledge of TBI in childhood to optimize management. And that is not even mentioning the considerable anatomical differences. There can be little debate that children are indeed very different.

OVERVIEW

Anatomy and physiology in children develops over several years to gradually assume the adult form. We need to be aware of these differences to prepare for common problems in childhood TBI.

CRANIAL AND SPINAL ANATOMICAL DIFFERENCES AND IMPLICATIONS FOR TREATMENT

Relative to the size of a child's body, the head is large and heavy, balanced on a neck poorly supported by weak muscles and ligaments, and so both head and cervical spine are easily injured. Biomechanical maturation of the spine is a progressive process that only starts to resemble the adult spine after age 8–9 years old. Epiphyses fuse at different times and are easily mistaken for fractures. The pattern of injuries is determined by these progressive changes. Most spine injuries in children occur in the cervical region; in younger patients, these are more often subluxations or dislocations, more often in the upper cervical spine, and more often associated with neurological injury (1–9). The fulcrum of movement descends from the upper cervical spine in young children, where C0–C2 injuries predominate, to progressively lower in the subaxial spine as they grow, when mid- to low cervical injuries become more common. The craniocervical junction is most vulnerable to injury and instability in young children because the articulations are more susceptible to movement than in older children, the ligaments and paraspinal muscles are weaker, and the dentocentral synchondrosis between the odontoid and C2 body are yet to fuse (5, 10). Congenital abnormalities of the dens and the atlas also increase susceptibility to injury. Careful attention must be paid to the lowermost axial images of the initial head computed tomography (CT), and the radiographer must ensure that craniocervical junction is well imaged: ligamentous injuries are common between C0 and C2 and are often manifest by retroclival hematomas (11, 12) (**Figure 1**).

In pediatric spinal injury, four patterns tend to predominate: fracture with subluxation, fracture without subluxation, subluxation without fracture [purely ligamentous injury and spinal cord injury without radiographic abnormality (SCIWORA)] (5). SCIWORA is peculiar to children and is of particular concern

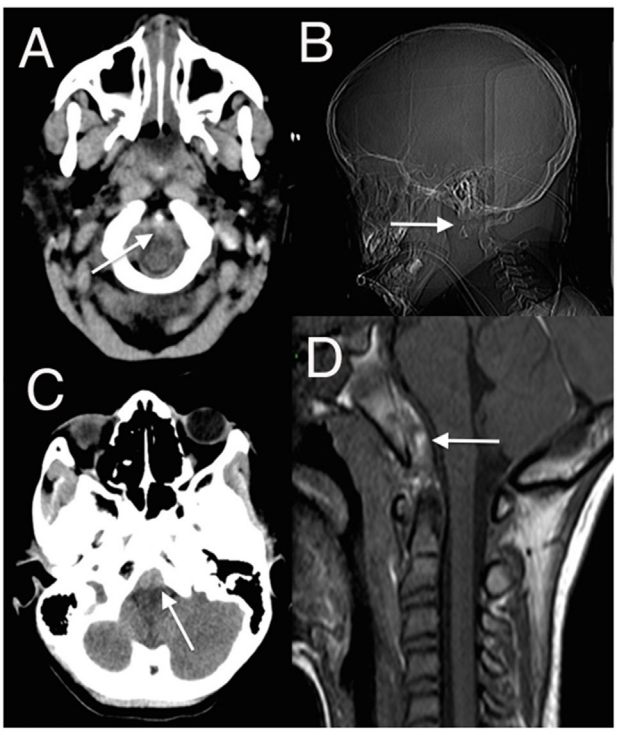

FIGURE 1 | **(A,C)** Axial head computed tomography (CT) scan with low posterior fossa cuts revealing the retroclival hematomas anterior to the lower brainstem (arrowed); **(B)** CT surview showing atlanto-axial dislocation (arrowed); **(D)** sagittal T1 magnetic resonance imaging (MRI) showing retroclival hematoma (arrowed) better demonstrated on the subsequent MRI (13) (modified).

because, by definition, radiographs are normal (14, 15). It reflects the easy deformation of the cervical spine with external loading and the risk to underlying neural structures. Several factors cause the cervical spine in children to be weaker and thus more easily deform: weaker cervical ligaments and paraspinal muscles, increased water content of intervertebral disks, unfused epiphyses, shallow facet joints, anteriorly wedged vertebral bodies, and undeveloped uncinate processes (16–23)—these all contribute to a more malleable spine that puts neural structures at risk, even without bony injury evident on radiographs. A high index of suspicion must be maintained, and magnetic resonance imaging (MRI) should promptly be done to investigate any signs of retroclival blood or long tract findings unexplained by the head injury (**Figure 1**). In awake patients, five clinical criteria have a high negative predictive value for significant spinal injury: normal alertness, absence of midline cervical tenderness, no focal deficit, no intoxication, and no painful distracting injury (9).

The head and the brain are fundamentally different to adults physiologically and anatomically. In the newborn and infant, the head is disproportionally large and gradually assumes the head:body ratio of an adult over several years. Growth is particularly rapid in the first few years of life. At birth the brain is about 25% of the adult size even though body weight is about 5%; about half of the postnatal growth of the brain occurs in the first year or two; the ratio of head and neck length to body length (about 25%) in infants is almost double that of adults, and this is a continuum from gestational changes (24, 25). The disproportionally greater weight of the head also affects the movement of the head when a child falls or is struck by a moving object (26).

The skull also undergoes considerable changes with age. Fontanels and sutures close at different times. At 2 months of age, the posterior fontanel is usually closed, and by 12–18 months, the anterior fontanel is closed. Open sutures and fontanels allow some buffering of ICP, especially if intracranial volume increases slowly, but only to some extent. In trauma, intracranial volume can increase rapidly, and so the increased compliance may be rapidly exhausted. Also, normal ICP in the very young is considerably lower than in adults, as is BP, so small increases in ICP may have significant adverse effects.

The calvarium is thin in young children; this, with the sutures and fontanels, allows for easy deformation, with or without fracturing, under external pressure (27–30). Diastatic skull fractures may also occur in children (31), where an unfused suture diastases as a result of direct trauma or deformation, and sometimes with raised ICP. Because of the pliability of the skull, a linear fracture may represent significant underlying parenchymal injury sustained by marked deformation at the time of injury despite little evidence on the head CT. This makes growing skull fractures a unique feature of young children (32–35). At the moment of impact, the deformed bone and fractured edges tear the dura. Soft tissue interposes between the fractured edges which then do not heal. The pulsatility of the brain and the growth of the cranium then combine to increase the fracture size over time, which further retracts the dural edges in a vicious cycle. So, surveillance for growing fractures is important and these require surgery. On the other hand, closed depressed, "ping-pong," fractures are common in very young children and can often be treated conservatively. They often mold to normality over a few months.

The thin skull may be a challenge for ICP monitoring—often surgeons are reluctant to use bolt systems or even measure ICP in the very young (36). If bolt systems are used in young children, the skull thickness must be measured on the head CT and the bolt thread adapted accordingly. Alternatively, the monitor can be tunneled. The young age of a patient should not be a reason not to monitor ICP.

If the dura is intact, small skull defects often heal well due to the osteogenic potential in childhood bone; however, resorption rates after bone flap replacement are higher in young children (37), especially when there is a significant delay in the bone being replaced. The growing head size and pulsatile nature of the brain contribute not only to this risk but also to the problems of cranioplasty using foreign material (37, 38). Split calvarial grafts are ideal in this situation but unfortunately the underdeveloped medullary layer makes this difficult in children under the age of 3.

Basal skull fractures are common, especially in crush injuries, in which release fractures may occur diagonally across the skull base. These must raise suspicion of an injury to the carotid artery (39) in the canal, especially when running into the sphenoid bone, and may warrant MR angiography. The vessel may be occluded by dissection and/or thrombus and this may be clinically silent in children if the crossflow through the circle of Willis is adequate. Even if the occlusion is asymptomatic, it is important to diagnose this because of the potential for thrombus extension.

At the mild end of the spectrum, decision-making about head CT in children is compounded by the greater sensitivity of the developing brain to the effects of radiation, in terms of both cancer-inducing potential and cognitive development. Therefore, several sets of decision rules have been evaluated to rationalize the indications for head CT to limit over-investigation (40). Even if resources were not a problem, the solution is not as straightforward as lowering the threshold for MRI. Children under the age of 8 years old usually need sedation or general anesthesia for MRI, which carries relatively low risk, but risk nevertheless. Also, there is growing concern of the effects of long and cumulative anesthetics on the developing brain, although this remains controversial (41).

The radiological pattern of pediatric TBI shows some differences to that of adult TBI (42). In severe pediatric TBI, diffuse injuries are more common than the focal injuries and contusions of adult TBI. In diffuse injuries, the scan looks relatively benign but the patient is in deep coma. A contusion in the midbrain is not uncommon as a manifestation of significant injury that may be subtle on head CT; MRI demonstrates the lesion more clearly. Patterns of injury are also determined by the mechanical properties of the brain tissue, which in children is stiffer than in adults (30). Non-accidental injury ("shaken baby syndrome") is peculiar to young children (43, 44) and is beyond the scope of this article but is an important specific pathophysiological entity to be aware of. The pathophysiology, radiology, clinical presentation, and outcome are very different to accidental injury in many ways, and this requires separate consideration.

Discrete hematomas in children are less common than in adults but of course do occur. Epidural hematomas in children

are somewhat different to those in adults (45–47). The middle meningeal artery is not as incorporated into bone as in adults, but epidural bleeds from the edges of a fracture easily lead to hematomas. They occur in a wider variety of locations (**Figure 2**), in part because these are often due to venous rather than arterial hemorrhages (13). Fractures in the occipital and suboccipital regions are particularly concerning because of the risk of a posterior fossa hematoma, which rapidly causes brainstem compression as well as hydrocephalus by fourth ventricular and aqueduct obstruction (48–50). Subdural hematomas (**Figure 3**) are associated with more severe injuries to the parenchyma, cortical veins, and venous sinuses (51–53). Associated arterial and venous infarcts are not uncommon. Non-accidental injury must also be considered in infants where there are bilateral subdural collections, particularly of differing ages (28, 54); however, one must also keep in mind that there are other medical and procedure-related causes of subdural hematoma (53, 55–58).

SYSTEMIC ISSUES AND IMPLICATIONS FOR TREATMENT

Several systemic anatomic and physiologic differences are relevant when managing head trauma in children. Young children are at particularly high risk of airway obstruction. Their tongues are relatively large for their oral cavity, as are the soft palate and soft tissues of the mouth and the epiglottis, which is relatively longer and stiff. They also have a larynx that is higher and more anterior, a cricoid ring that represents the narrowest point of the airway, and a shorter trachea that bifurcates higher (59, 60). The trachea has a small diameter and is compressible, so even small changes in diameter or foreign bodies can rapidly lead to airway compromise; frank respiratory embarrassment is accelerated by their reduced functional residual capacity and higher metabolic requirements per weight (61). Because they have a large occiput, their necks flex easily when lying supine, which may contribute to airway compromise. The chest wall is cartilaginous and more easily deformable; rib fractures are unusual but lung contusions are common and may be severe despite little external evidence of injury (61, 62). Because of their small lung volumes it is easy to unintentionally hyperventilate children during resuscitation (especially with manual ventilation) and so hypocapnea is common, which may be particularly detrimental at a time when CBF is already reduced. Abdominal injury and gastric distension easily constrain breathing because children are diaphragmatic and abdominal breathers.

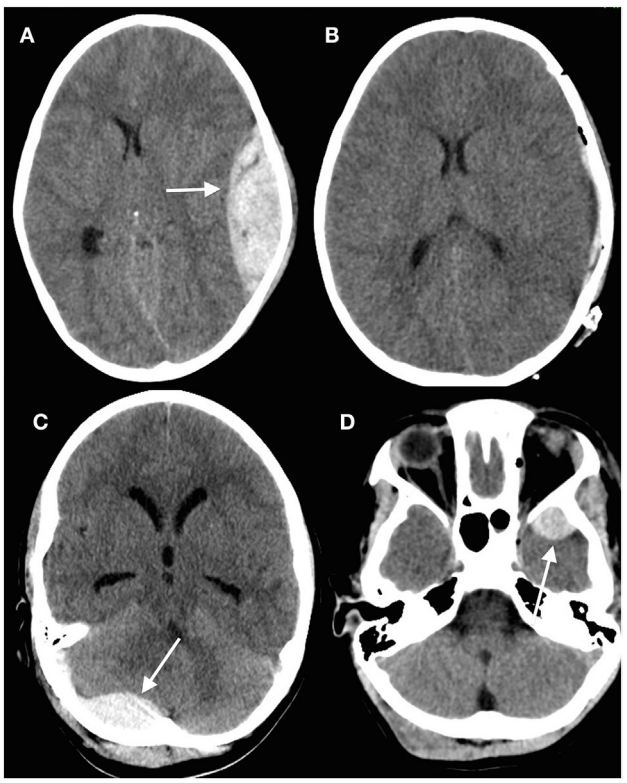

FIGURE 2 | Epidural hematomas occur in a variety of locations. **(A)** Head computed tomography (CT) showing a typical convexity epidural hematoma in a child; **(B)** evacuated hematoma in the same patient; **(C)** posterior fossa epidural hematoma (arrowed) underlying a suboccipital fracture; **(D)** epidural hematoma anterior to the left temporal tip (arrowed) (13) (modified).

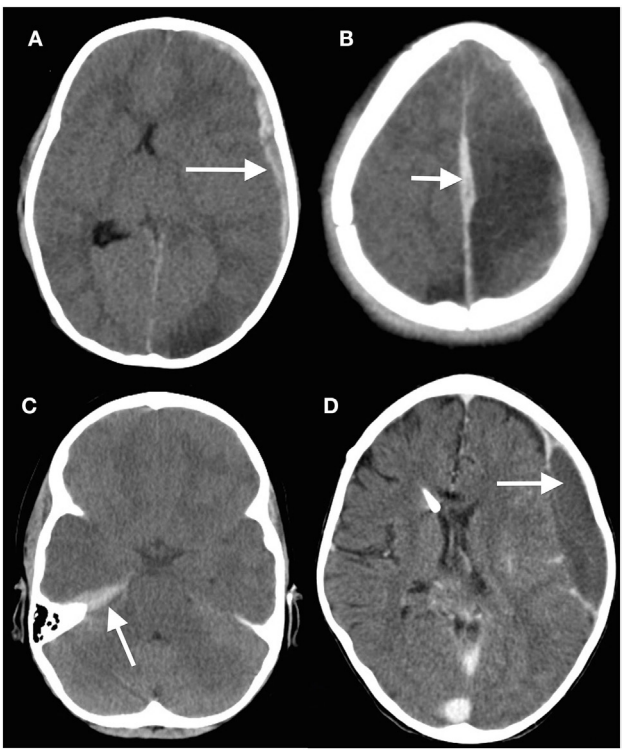

FIGURE 3 | Subdural hematomas in children. **(A)** Head computed tomography (CT) scan showing a typical acute subdural hematoma with a hypodensity in the ipsilateral posterior cerebral artery territory; **(B)** interhemispheric subdural hematoma (arrowed) with adjacent venous hypodensity; **(C)** subtle subdural hematoma situated on the tentorium beneath the temporal lobe; **(D)** minor knocks to the head can easily cause a subdural hematoma (arrowed) in children with ventriculoperitoneal shunts, especially if there is a degree of overdrainage from the shunt (13) (modified).

Insensible fluid losses and heat loss is common, and so hypothermia easily occurs, especially in the very young: neonates and infants have body surfaces as much as three times that of an adult, with proportionally large heads for their body size. Bones break or are deformed easily and so polytrauma is common—long bone fractures, chest wall injuries, and injuries to underlying intrathoracic and intra-abdominal organs. Rapid low dose whole body radiographs may reduce the overall radiation burden in children who have suspected polytrauma and are a useful rapid screening tool (63). Injury to solid organs is relatively common because children have proportionally larger organs (which are also closer to each other), less intraperitoneal fat and weaker abdominal musculature as protection (62, 64, 65). Focused abdominal ultrasound has a high specificity for detecting hemoperitoneum (66). Fortunately, most abdominal injuries in children can be treated conservatively (62, 67).

Blood pressure control is pivotal both in the intensive care unit and in the operating room. During surgery, anesthesiologists often maintain relatively low BPs to reduce blood loss. However, this may compromise perfusion, both in handled tissues and a swollen brain. Given the importance of BP control in surgery, there is surprising variability in how hypotension is defined. For some, it is a decrease of more than 20–30% from the baseline systolic blood pressure (SBP), others use variable normograms (68). For neurosurgical patients though, we need to maintain perfusion not only of physiologically normal tissue but also of tissues penumbral to a lesion. Hypotension tolerable in normal children may cause harm in children with TBI. At the same time, high BPs cause unnecessary bleeding, as well as brain swelling if autoregulation is impaired.

Hypotension must be avoided in TBI—there is a similar association between hypotension and poor outcomes in childhood TBI as in adult TBI (69). But there are several additional challenges: as stated above, BP normograms are often not used, and the circulating blood volume changes dramatically with age (70, 71). In young children, this is a small volume—"minor" blood losses can have major clinical implications. Neonates have a circulating blood volume of approximately 85–90 ml/kg, infants 75–80 ml/kg, older children 70–75 ml/kg, and adults 65–70 ml/kg. Therefore, loss of 50 ml in a 3 kg child represents almost 20% of their circulating blood volume. The issue of BP maintenance is discussed further below.

BRAIN PHYSIOLOGY AND MONITORING

Cerebral Compliance

Open fontanels and unfused sutures allow for increased cerebral compliance in young children, but only to a point. When intracranial volume increases rapidly, as in trauma, raised ICP is as important an issue in young children as it is in older children and adults, perhaps even more so because of the low normal range of ICP in this age group. Still, ICP monitoring is used infrequently in these children (36). Cerebral compliance is also affected by CBF and volume, and the ratio of cerebrospinal fluid (CSF) volume to brain, all of which are age-dependent.

Given the differences in CSF-brain ratios, clinicians must be familiar with typical imaging across the age ranges—it is easy to misinterpret the likelihood of raised ICP because of these differences. The CSF-brain ratio reflects the balance between brain tissue and CSF in the ventricles and subarachnoid cisterns of the brain. Although this has not been formally quantified across the age range, radiologists and pediatric specialists are aware of the differences between very young children, older children, and adults with respect to the amount of intracranial CSF that is expected, reflecting the growth of the brain from the neonatal stage through childhood and the development of atrophy with age in adults (72, 73). As in adults, the patency of basal cisterns is an important indicator of ICP (**Figure 4**), but it is by no means absolute (74). Just because the cisterns are open does not guarantee that ICP is normal. Also, brain swelling can change rapidly over time, especially in children, in whom cerebral blood volume changes are a common cause of increased ICP. Therefore, what the scan looks like at one point in time may bear little semblance to what it looks like several hours later.

Cerebral Blood Flow

Understanding CBF is more challenging in pediatric TBI, in part because hyperemia is reported to be a frequent cause of raised ICP (75) and because normal CBF varies with age. These changes with age are probably the reason that diagnosing hyperemia in children is not as straightforward as it would seem (76–78), so it would be easy to misinterpret what may well be normal for a particular age group. It is also difficult to study; the data we have currently heavily depend on the tools used to determine CBF. These have included ultrasound-based techniques, MRI, and positron emission tomography (PET). The conditions under which the study is performed also markedly affect the outcome. Studies are difficult in children and so sedation or anesthesia is often used, both of which of course affect CBF. To illustrate, across three studies in children, using different techniques, the following results are reported for average total CBF: 760/781 ml/min (girls/boys, respectively), 1,101 ml/min (girls and boys), and 1,538 ml/min (girls and boys) (79–81).

Still, there are a few things we know. Rapid changes in metabolic demand in the early years follow cortical development,

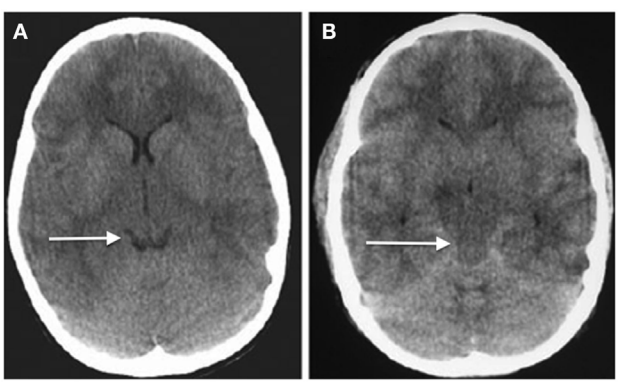

FIGURE 4 | Axial head computed tomography (CT) scans showing **(A)** relatively normal looking hemispheres with open basal cisterns (arrowed, "smiling brain") and **(B)** diffuse severe swelling and obliterations of the cisternal spaces (arrowed) (74) (modified).

progressive myelination, and synaptogenesis. CBF is lowest at birth and in neonates, peaks at ages 3–7, and then progressively decreases to adult levels (78, 80–83). CBF volume shows similar changes. The sharpest increase is in the first 6 months of life; this continues over the next 3 years at a slower pace. In 3-year olds, the CBF volume is ten times greater than in the newborn (84). CBF volume in neonates is 70 ml/min and about 700 ml/min in 3-year-old children (80). In a PET study of children, regional CBF was 140–175% of adult values for children between the ages of 3–7 years, although cerebral metabolic rates of oxygen were less markedly different (100–120% of adult values) (83). When normalized for brain volume, which of course changes with age, global cerebral perfusion (total CBF divided by brain volume) reaches a peak of around 2.5 times that of adults between the ages 3 and 4 (81, 85).

But it is not just the baseline differences that matter. Pediatric brain metabolism also responds differently to activation. One study of 8–12-year-old children showed a similar percentage increase in CBF after activation but a greater increase in *absolute* flow when compared with adults (86). This may account for some of the dynamic changes in ICP in children with TBI despite minimal stimulus. Cardiovascular changes with age must also be considered. A higher metabolic rate in children is associated with higher cerebral and cardiac indices. A greater proportion of the cardiac output goes to the brain in children, in keeping with the higher cerebral metabolic rate—the fraction of cardiac output to the brain is more than twice that of adults (81). All of these factors affect the hemodynamic changes in ICP and CPP in children with TBI.

So, when determining what CBF is in normal and pathological states, whether ischemic or hyperemic, it is clear that several age-related phenomena must be considered. Unfortunately, the evidence base is lacking because children are inherently more difficult to study than adults, and there are few bedside tools that can be applied to children with TBI that provide good data. Transcranial Doppler (TCD) is an example of such a tool. It is commonly used but has significant limitations, in particular because only flow velocity in the basal vessels is determined. High flow velocity must be distinguished from what is normal in children based on age and what may be vasospasm. Little is written about posttraumatic vasospasm in children and unfortunately the Lindegaard ratio is not always reported, an important factor to consider when interpreting high cerebral blood velocities values by TCD (87, 88). This is the ratio between the flow velocity in the middle cerebral artery over the flow velocity in the internal carotid artery. In adult patients, it is reported that to diagnose vasospasm the flow velocity in the middle cerebral artery should be greater than 120 cm/s, and the ratio should be greater than 3 to diagnose vasospasm. To date though, this has not been validated in children. In general though, vasospasm appears to be less common in severe pediatric TBI than in adults (89); however, one study reported vasospasm in as much as one third of children (90). These figures are based on TCD parameters developed in adult subarachnoid hemorrhage patients and so may not apply to children. Much work needs to be done using bedside tools to guide hemodynamic and metabolic changes by the bedside.

Intracranial Pressure

Although it is clear that ICP is injurious to the brain as a secondary mechanism, causing brain shift and brain ischemia, with often an inverse relationship with perfusion of the brain (**Figure 5**), there is ongoing controversy about ICP thresholds for treatment in adult patients, recently aggravated by the South American trial of ICP monitoring in severe TBI. Although the trial has been criticized and it is currently recommended that

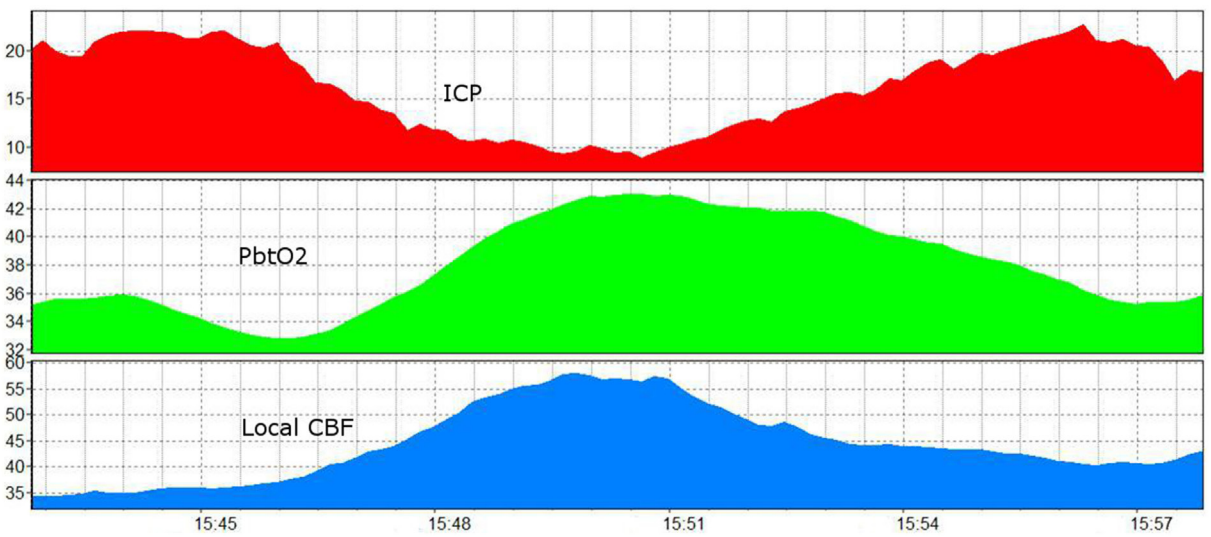

FIGURE 5 | A 15 min recording showing the influence of increased ICP and ICP reduction on brain oxygenation and brain perfusion (92) (modified). ICP, intracranial pressure (red, in mmHg); $PbtO_2$, brain tissue oxygen (green, Licox, in mmHg); CBF, local cerebral blood flow (blue, Hemedex local tissue monitoring, in ml/100 g/min).

existing protocols for ICP monitoring should not be changed (91), there is no doubt that greater uncertainty has crept into the management of raised ICP in trauma. Arguably, this trial may have been unsuccessful because there was a singular focus on ICP, with little consideration given to the complexity of cerebral dynamics. There are several different causes of increased ICP but recommended treatment protocols are insensitive to these. Therefore, ICP treatments may well be inappropriately applied. Furthermore, given the heterogeneity of causes of increased ICP as well as inter-individual differences, ICP thresholds for injury likely vary across patients. Lastly, all ICP therapies have adverse consequences and so the risk-benefit ratio should ideally be determined for each situation.

These are some of the obvious controversies and uncertainties in managing ICP in adult TBI. To this is brought further unknowns for pediatric TBI. Much of the pediatric recommendations for TBI care are extrapolated from adult studies; there is very little pediatric-specific evidence. This is why the recommended ICP treatment threshold is also 20 mmHg (93), despite general awareness that normal ICP is lower in children and the fact that raised ICP in children behaves differently. To date, there are no age- or cause-specific recommendations for thresholds or therapies.

A key starting point is knowledge of normative ICP values in children, for which there are surprisingly little data. Much of the existing knowledge derives from examination of CSF opening pressures from lumbar punctures. A series of these studies from one institution (94–97) sought to determine ICP thresholds in children. They examined children aged 1–18 years old who apparently had no condition that would increase ICP: 1,066 children were screened, the authors enrolled 472, and after exclusions investigated 197. Their results suggested that the upper limit of normal ICP for children was 28 cm H_2O (20.6 mmHg). The opening pressures were normally distributed and showed a mean of 19.6 cm H_2O, with 10th and 90th centiles of 11.5 cm H_2O and 28 cm H_2O, respectively. The authors then went on to study children who had fundoscopic evidence of optic nerve head edema and reported their results in keeping with their previous findings.

Cerebrospinal fluid opening pressures are commonly used as a measure of ICP, ever since it was first described in 1891 by Quincke (98). Still, there is no general agreement that it accurately reflects ICP, especially in diseased states. Cartwright et al. studied 12 children (mean age 8.5 years) who were being monitored with an intracranial device (Camino, Integra Neurosciences) and who underwent lumbar puncture. The values were quite discrepant ($p < 0.001$): mean ICP from the Camino monitor was 7.8 mmHg compared to 22.4 mmHg from the lumbar puncture. The authors suggested that lumbar CSF opening pressures significantly overestimated the true ICP (99). The lumbar puncture technique was similar to that used elsewhere. The case mix included patients referred for evaluation of craniosynostosis and idiopathic intracranial hypertension (there were no TBI patients). This affects the generalizability of their results, but such is true also for studies of "normal" children—we generalize physiology to pathological conditions. Lumbar opening pressures are affected by several variables—including technical factors such as patient positioning and sedation, as well as pathology-related factors. Furthermore, a once-off determination of ICP does not reflect compliance, dynamic ICP changes, or ICP behavior after stimulation. Lastly, the ICP tolerated in the normal physiological state may not be tolerable if there are already factors reducing tissue perfusion or if physiological mechanisms such as pressure autoregulation and flow-metabolism coupling are impaired.

So even if these numbers are accurate for normal children, can they be applied to a swollen, ischemic brain? At what point is brain perfusion impaired? Is the cause of increased ICP relevant to this decision? Accumulating evidence suggests that the relationship between ICP and perfusion of the brain is complex (92). In children, ICP often changes rapidly from 1 min to the next. Often, the cause of this appears to be vascular in nature. In keeping with this, simultaneous increases in ICP and brain oxygenation (or CBF) are often observed, but only to the point where the rise in ICP (presumably from increased cerebral blood volume) appears to have an adverse effect on tissue perfusion, at which point the relationship changes. The point at which this happens is variable and there may not be a specific threshold consistent across all children. This phenomenon, along with age- and cause-specific differences, produces heterogeneity and explains the observation that the relationship between ICP and brain oxygenation is weak when pooled across all patients, even though they may be tightly linked in episodes in individual patients (92). Indeed, this may represent part of the interindividual variability that confounds many of our treatments and leads to negative studies, in large part because not all patients respond the same to treatments, or in fact need that particular treatment at all (100). This raises several questions: should the threshold for ICP treatment be different if the cause of increased ICP is increased blood flow, i.e., if perfusion is not compromised can we be permissive about ICP higher than our traditional target? Conversely, if perfusion is affected at ICP thresholds less than 20 mmHg, should we intervene earlier? These have been some of the questions driving the use of multimodality monitoring to make individualized, or at least better, decisions at the bedside (101).

BP and CPP

If determining ICP treatment thresholds in children is complicated, attempting the same for CPP is worse. CPP depends on ICP and mean arterial pressure (MAP), and so to the uncertainty around ICP are added the variables about changing normative BP across the age range and the complexities of pressure autoregulation. It would appear reasonable that adequate CPP should be age-based but no such recommendation exists. For the ideal normative BP range, sex and height should also be considered but rarely are. Even the definition of hypotension is surprisingly variable (see above). Most definitions rely on SBP, not MAP. Various estimations based on age exist, particularly so for resuscitation. One common estimation for SBP at the 5th centile (at 50th centile for height) is 2× age in years + 65; for MAP this is adjusted to 1.5× age in years + 40. The 50th centile for systolic BP is calculated as 2× age in years + 85; and for MAP, 1.5× age in years + 55 (102). The Pediatric Advanced Life Support guideline is slightly different: hypotension is defined as SBP less than the following thresholds: 60 mmHg for neonates, 70 mmHg for infants (1–12 months), (2× age in years + 70) for children

aged 1–10, and 90 mmHg over the age of 10. Useful tables can be found from an analysis of data from 60,000 children in the National Center for Health Statistics database (102), in which the authors charted 5th–95th centiles accounting for age, height, and sex. They also compared their definitions of hypotension to that of other sources (102).

These recommendations about BP management are for generally ill patients, not for those with a brain injury, for whom optimal BP control may be substantially different. To start, MAP is more useful than SBP and ICP must be known to calculate CPP. Some institutions prioritize CPP above ICP, arguing that CPP is the ultimate driving force for perfusion of the brain. This may be so but increased tissue pressure can decrease local tissue perfusion regardless of CPP and impaired autoregulation may exacerbate the risks of chasing a target CPP.

Adult practice in various centers has ranged from aggressive CPP management (103) to minimized CPP targets (104). What is clear is that chasing higher CPP targets increases the risk of lung pathology due to aggressive fluid and inotrope administration (105). There is a growing consensus that an optimal CPP varies substantially between patients. But how best to optimize CPP individually remains uncertain. One school of thought argues that an optimal CPP can be determined from passive correlation analysis between BP and ICP (as a proxy of blood volume) and in so doing develop a pressure reactivity index, which allows calculation of an "optimal CPP" target at which autoregulation is most active (106). However, just because the CPP is optimal with respect to that measure, it does not necessarily follow that a patient needs that CPP for adequate brain perfusion. Others use various ancillary measures to determine the adequacy of blood flow to the brain such as microdialysis and brain oxygenation.

The published guidelines for children suggest a CPP threshold of 50 mmHg in older children and 45 mmHg below the age of 2 (93), but the evidence base for this is weak. No age-based recommendations exist for children. Autoregulatory status likely affects an optimal CPP, but this is rarely used (see below). Similarly, measures of brain perfusion adequacy are also rarely used (see below). Importantly, current data suggest that patients managed according to the published guidelines still commonly (around one third) experience episodes of very low brain oxygenation despite adequate adherence to targets for ICP, CPP, and systemic oxygenation (107).

There is also ongoing discussion about whether MAP should be zeroed at the level of the head or the heart. This problem is arguably exacerbated in children because the difference in vertical height between the head and the heart when patients are managed with elevation of the head of the bed is more variable in children because it is influenced by the length of the patient. However, some argue that these differences are of little clinical consequence because perfusion of the brain is subject to the siphon effect—it is in essence part of a closed loop of perfusion from the heart to the brain and drainage back to the heart (108, 109).

Autoregulation

About 30–40% of children with severe TBI develop impaired autoregulation in the acute setting (110–112). When autoregulation is impaired patients are at greater risk at both lower and upper ranges of BP. Therefore, it is unsurprising that impaired autoregulation is associated with worse outcomes (113). Currently, there are no recommendations of how autoregulatory capacity should be considered in the management of pediatric TBI.

Few studies have examined autoregulation in pediatric TBI, either as dynamic testing or pressure reactivity index estimation (110–118). It remains uncommon that autoregulation is measured as part of clinical care in pediatric TBI, despite the impact this has on the relationship between BP and cerebral hemodynamics, including cerebral blood volume (and therefore ICP) and cerebral perfusion. It would be sensible to have a measure of autoregulatory capacity to assist decision-making. BP can be titrated against some measure of perfusion adequacy while aware of its influence on cerebral blood volume, such as combining brain tissue oxygen ($PbtO_2$) and ICP monitoring. If autoregulation is impaired, augmenting BP to achieve CPP simply increases ICP. At best it is of little help, at worst it may be detrimental. Therapy should be focused on ICP reduction. If autoregulation is intact, there is greater capacity for BP augmentation to benefit CPP. In some patients, this may actually result in some reduction of ICP because of the vasoconstrictive response.

Carbon Dioxide Reactivity

Carbon dioxide (CO_2) reactivity is a robust and well described response of cerebral arterioles that is usually preserved in the injured brain (119). Occasionally, it may be impaired in the first few days after injury (120). The mechanism is of great clinical relevance because of therapeutic potential as well as unintended changes in CO_2. We have to be particularly aware of this in children, where the mechanism appears stronger than in adults (78). Unintended changes in CO_2 are particularly common in children because of their small lung volumes—for this reason, accidental hyperventilation is common during resuscitation. Because hypocapnea vasconstricts cerebral arterioles, it decreases cerebral blood volume and therefore ICP, but at the cost usually of decreased CBF (in the normal physiological state anyway). This is particularly hazardous in the early phase of head injury, where CBF may be abnormally low. Conversely, a rise in CO_2 vasodilates cerebral arterioles, which may lead to increased perfusion, but also increases cerebral blood volume and therefore ICP. The subsequent increase in ICP may have a secondary negative effect on perfusion. This paradoxical effect may also be seen in hyperventilated patients with high ICP. Despite the fact that hypocapnea decreases arteriolar diameter, occasionally the reduction in ICP has a net beneficial effect on perfusion, at least until a certain threshold is reached. Beyond that, the effect likely reverts to expected reduction of perfusion associated with vasoconstriction as there is no further perfusion benefit of reducing ICP below that threshold. The key is not to assume how CO_2 will affect perfusion, but preferably to measure it.

In the past, hyperventilation was recommended therapy for raised ICP before a randomized controlled trial reined in this enthusiasm (121). The specifics of that trial, though, are worth reconsidering. Most importantly, it examined a very specific application of hyperventilation, namely prolonged, severe,

and not targeted to a specific ICP crisis. The treated group was hyperventilated to a mean arterial CO_2 of 25 mmHg; they fared worse at 3 and 6 months, but no differently at 12 months. The practice of hyperventilation declined after this trial, but it is still considered an option in current guidelines, under some sort of perfusion monitoring. Unfortunately, this has not been tested in large populations. The practice of hyperventilation though (uncontrolled by perfusion monitoring) is still common—in the controlled trial of ICP monitoring referenced above, hyperventilation was used in 60% and 73% of the ICP monitoring group and imaging-clinical groups, respectively. Manipulation of CO_2 in a controlled environment may still be of value, but we need to examine its use for more limited time periods, controlled with perfusion monitoring, and as a strategy to break an ICP crisis. However CO_2 manipulation is used though, it must be remembered that the effects are temporary.

Brain Oxygenation Monitoring in Children

Methods of brain oxygen monitoring (122) have not been extensively studied in children. Of the various methods, invasive tissue monitoring (PbtO2, or partial pressure of brain tissue oxygen) and near-infrared spectroscopy (NIRS) have some data in pediatric TBI. NIRS has been evaluated more commonly in neonates and cardiac patients; there are fewer studies relevant to TBI. There are currently more studies of PbtO2 (107, 110, 123–128); although the number remains small compared to those in adults. Data from the largest series (126) is consistent with the adult experience in terms of thresholds related to outcome, although one report suggested a higher threshold may be more predictive for a favorable outcome (128). Published data and clinical experience also confirm its value as an ancillary test in patients diagnosed with brain death and as predictors of mortality and functional outcome (123, 126, 129). Whether treatment directed at maintaining PbtO2 improves outcomes is yet to be determined in adults and children.

Importantly, the relationship between PbtO2 and ICP is mixed. Although in some patients, there is often a clear and strong negative correlation, when averaged over several patients, the correlation is poor (92) for several reasons—variations in autoregulation, different responses to CO_2 changes (as discussed above), hyperemia, vasospasm, and electrophysiological events—all of which create complex relationships between ICP and brain perfusion.

CBF Monitoring

Cerebral blood flow monitoring is not used very often in pediatric TBI, in large part because the tools are not well developed (130). Spatially resolved techniques are of limited application because of the dynamic nature of pediatric cerebral hemodynamics. Local CBF monitoring shows good temporal resolution but poor spatial resolution, which is true for all forms of catheter based monitoring. The most frequent reports in children involve TCD recordings, which of course measure flow velocity in the basal vessels of the Circle of Willis, not true flow. Still, it has applications that may be of use, including as a tool to determine flow changes in autoregulation tests, detect vasospasm and perhaps as a non-invasive measure of ICP (131). Other limitations of TCD are that it is operator dependent and long-term monitoring is difficult because changes in the insonation angle affect recorded values. O'Brien has published reference values for critically ill and sedated children (132).

The Bowman perfusion monitor (Hemedex) uses a thermodilution method to determine local CBF, but it has not been widely used (133). Imaging of blood flow varies from perfusion CT to PET imaging. These may be valuable in research and for point-in-time assessments of brain perfusion (excellent spatial resolution) but are less helpful for managing the dynamic nature of brain injury (poor temporal resolution). Brain physiology is dynamic in the acute phase and responds differently over time due to changing systemic physiology. Because of radiation concerns, xenon CT is rarely used, but previous studies produced some insights. Adelson et al. studied CBF in 95 children with xenon CT and found that unfavorable outcomes were associated with reduced mean CBF (134). When CBF was less than 20 ml/100 g/min in the first two days postinjury, outcome was universally poor. Disturbed CO_2 vasoreactivity was also associated with poor outcomes.

Brain Metabolism

Brain metabolism in children changes with advancing age. It depends on progressive myelination and synaptogenesis and drives the substantial changes in CBF, especially in the first 8 years of life (83, 84). Cerebral metabolism of glucose starts at low rates of around 60% of adults values at birth, but rapidly accelerates to over 200% adult values by age 5 before slowly decreasing to adult levels through adolescence (135). As yet, it is unclear what implications this has for treatment, including the most basic aspect of supportive care, nutrition. Currently, there are no clear recommendations on when and how to feed after severe TBI in children (93), and current practice variation across centers is wide (136).

To date, there have been little data on imaging metabolism in children, in part limited by the "snapshot" methodology, the need to move unstable patients, and radiation exposure in children (137). Continuous local monitoring of basic parameters of metabolism is possible through microdialysis but has been rarely used in children. This was first described in adult TBI in the early 1990s and although clearly a useful technique for investigating brain metabolism, it has not achieved widespread utilization as a clinical tool (138). It remains a valuable tool in research-led environments, but wider adoption is likely limited by costs and effort required to run an effective program in which catheters are placed, vials changed regularly by the bedside and analyzed, and clinical decisions made on the basis of chemical changes. Little has been published in children. Tolias et al. reported a small series of children with severe TBI who underwent microdialysis monitoring, but concentrated on glutamate (139, 140). Preliminary metabolic data from microdialysis in children are in keeping with adult data (141). In this cohort of 22 children elevated lactate–pyruvate ratio was associated with mortality, poor clinical outcome, and low brain oxygen; and glucose decreased at lower CPPs.

Management of Fluids, Hemogobin, Glucose, and Temperature

As with adults, similar controversies and uncertainties exist in the pediatric literature for fluid, hemoglobin, and glucose

management, but with the confounding of having less evidence. As is usual, wide differences exist in practice at individual institutions. Normal saline is used routinely to avoid hypotonic fluids in patients with brain swelling. If CSF is being drained, sodium losses must be calculated and replaced. Some centers avoid all dextrose in fluids in the early phase of head injury, and there are variable times at which nutritional support is started (136). At our institution, we start nutrition early and do not restrict glucose in intravenous fluids but watch the systemic glucose closely. Glucose control has not been studied exhaustively in pediatric TBI. Although hyperglycemia is known to be associated with poor outcome, tight glucose control has not been studied in children with TBI. In the adult experience (142), brain glucose correlates with serum glucose, but there are discrepancies in individual patients at various times; therefore, there is presumably a greater risk of neuroglycopenia in patients who have tight serum glucose control without knowledge of brain glucose. Neuroglycopenia may be a greater problem than systemic hyperglycemia, and so caution is advised when considering tight glucose control.

Most centers use relatively conservative guidelines for initiating blood transfusion in critically ill children, and there are no specific data recommending a different practice for TBI, although the concern about brain ischemia and hypoxia is greater in these patients. We studied changes in PbtO2 before and after blood transfusion, controlling for all factors likely to influence any changes but were unable to determine any predictive factors (125). Similar results were found with transfusion for chronic anemia in children using NIRS (143). When we compared patients at the same stage post head injury, it appeared that transfusion was associated with an increase in $PbtO_2$ in most but not all patients in the first few hours after transfusion, but that this difference did not persist 24 h later. One retrospective study reported that blood transfusion was independently associated with worse outcome in children with TBI (144). However, even good multivariate models cannot fully control for differences in injury severity and disease complexity that may influence the decision to transfuse. Currently, we follow guidelines for transfusion triggers in generally ill patients but raise that threshold in children who have documented evidence of cerebral ischemia or tissue hypoxia.

THERAPIES

Unfortunately, there is less evidence on which to base therapies in pediatric severe TBI. Importantly, there is an ongoing multicentre comparative effectiveness trial of therapies in severe pediatric TBI with ICP monitoring that may add much needed data (145). Currently though, most recommendations are still not substantially different from the adult guidelines and are largely set at the level of an option (93). There is no evidence to support the use of hypothermia in children, despite ongoing arguments for the case in adults (146–149). Avoidance of hyperthermia, however, is widely accepted as a sound strategy. Hypertonic saline is preferred as a hyperosmolar therapy in children with severe TBI but practice is widespread, there is no standardization of use, and various formulations are used in different centers (93, 136). Long-term propofol is not used because of the concerns about fatal metabolic acidosis in children (150).

Decompressive craniectomy remains a controversial topic but the general sense is that younger patients, including children, may have greater potential benefit than in adults. Two randomized controlled trials in adults have not diminished the controversy. The DECRA trial (151) examined the use of craniectomy at a very low intervention (ICP greater than 20 mmHg for more than 15 min) in patients with diffuse injury only. The trial found that craniectomy did not benefit patients; however, their selection criteria do not generally reflect the situation in which craniectomy is commonly performed worldwide. Still it clearly established that craniectomy was not useful as a very aggressive early intervention at such a low threshold. The RESCUE ICP trial (152) included patients with mass lesions, and ICP greater than 25 mmHg for anywhere between 1 and 12 h despite stage 1 and stage 2 measures. Their results have been debated heavily since—the mortality rate was significantly lower in the surgery group but so was the occurrence of severe disability and vegetative state. Twelve months after injury and with rehabilitation, the proportion of survivors with independent function was greater in the surgical group, but the debate about what constitutes a good quality life continues. It is worth noting, however, that for ethical reasons, neither trial strictly examined craniectomy against pure medical management, as there had to be capacity for crossover. In the RESCUE ICP trial for example, almost one third of medically managed patients ended up with a craniectomy anyway.

How these results apply to children is still unanswered. The decompressive craniectomy studies in children have been single center observational trials (153–162) with the exception of one underpowered pilot study—27 children were randomized by Taylor et al. (163) and the surgery group appeared to have better outcomes. However, this was a pilot trial that was never developed further and the procedure—bilateral small temporal disk craniectomies with no dural opening—was dissimilar to the commonly employed techniques and produced only a very modest reduction in ICP. One of the problems with craniectomy in children is the high rate of bone resorption, particularly in the very young. This is further compounded by the fact that cranioplasty using foreign material is also more difficult in children with a growing skull (37). This must be evaluated with the other known complications of craniectomy including hydrocephalus (164).

DEVELOPMENTAL OUTCOMES

Better outcomes are usually reported for children compared to adults. However, there are several potential confounders in this. First, the adult cohorts often include significantly older patients, who are known to fare poorly. Second, the mechanisms and patterns of injury are different. Finally, assessment of outcome in children is made difficult by the lack of a stable baseline for comparison—children are in a developmentally accelerated phase (165). Unfortunately, various pharmacological therapies have shown promise in the laboratory but have failed to improve outcome after TBI, including most recently progesterone in the PROTECT III trial. As these trials are in adults, the data in children are limited.

Still, plasticity in children may aid recovery substantially in ways lost to adults. Enriching environments maximize this

potential: animal and human studies show greater cognitive improvements associated with dendritic arborization in stimulating environments (166). That bodes well for recovery, but the developing brain is a double-edged sword. The youngest patients are at highest risk of poor outcomes because of the developmentally immature brain.

Another factor worth considering is the increasing concern about long-term inflammatory processes that may develop even after mild head injury. Arguably, the younger the age at which the injury occurs, the greater the potential for cumulative injury to occur over many years. Much of the concussion literature dedicated to understanding the long-term risk of neurodegenerative disease after mild injury concentrates on adult patients, highlighting post-mortem findings of neurofibrillary tangles and tau protein deposition (167, 168). To date, we have limited longitudinal data for children. Arguably, when the brain is injured at a young age, these changes may develop over many more years and lead to secondary neurodegenerative diseases at a much younger age than the normal population.

Even anesthesia itself may pose a risk to the developing brain. A recent warning from the FDA has increased concern and confusion about the risk of exposure to anesthetics at a young age and stirred controversy about the subject (169). The data are far from conclusive as yet, but animal studies suggest that anesthesia may induce apotosis and interfere with neuronal differentiation, synaptogenesis, and network formation, ultimately having detrimental effects on neurocognitive development (41).

SUMMARY

Adult clinical services underestimate differences between adults and children. There is no doubt that anatomy and physiology in children relevant to central nervous system injury is profoundly different, and there are clinically important differences even within the childhood age range. So, pediatric services must contend not only with all the uncertainties and controversies that plague the management of adult TBI but also with all the different physiologic factors in a fundamentally different and changing population for whom ironically there is much less evidence. Better treatment protocols must be developed by limiting inappropriate extrapolation from adult studies and prioritizing pediatric-specific studies to guide clinical recommendations. In absence of this, the most vulnerable population will continue to receive second-rate care because of lack of evidence.

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The author confirms being the sole contributor of this work and approved it for publication.

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