CSF biomarkers for improved prognostic accuracy in acute CNS disease

Axel Petzold *

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Abstract

Providing an accurate prognosis in patients suffering from acute CNS disease is difficult. The extent and location of the primary CNS injury is crucial for mortality and morbidity. Secondary injury adds considerably to the CNS insult. The prognosis in an individual patient depends on the combination of primary and secondary CNS damage together with systemic complications. The clinical challenge of an accurate prognosis is therefore aided by both quantitative techniques, able to estimate the degree of CNS damage and qualitative techniques, which identify the site of the lesion. Here the contribution of cerebrospinal fluid (CSF) biomarkers to improving the prognostic accuracy is reviewed. Firstly current definitions in biomarker research are introduced. Secondly the role of the blood brain barrier is discussed. Thirdly the physiological and anatomical constraints of the CSF are summarised. Fourthly following an overview on CSF biomarkers, cell–type specific CSF biomarkers and more global CSF biomarkers for parenchymal CNS damage are reviewed in detail. The release of these biomarkers from dying cells is illustrated by a video of a laser–dissected neuron. Finally, the evidence for improving prognostic accuracy is summarised and recommendations are made for future biomarker research.

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*UCL Institute of Neurology, Department of Neuroimmunology, University College London; The Tavistock Intensive Care unit, The National Hospital for Neurology and Neurosurgery, University College London Hospitals (UCLH), Queen Square, London WC1N 3BG.

1 Introduction

Neurocritical medicine provides care to some of the most vulnerable patients. In most patients the level of consciousness is impaired to the level of coma, either because of the injury or secondary to deep sedation. Assessing these patients is clinically challenging. The clinical assessment is helped by standardised and validated scales targeted at the critically ill patient [1, 2, 3, 4]. A bad score on these scales implies severe brain damage and is a poor prognostic sign [3, 5, 6, 7, 8]. One problem in giving an accurate prognosis for an individual patient however is that some patients with a poor score make an unexpectedly good recovery, whilst others with a very good score suddenly deteriorate with subsequent poor outcome. The additional use of CSF biomarkers may help to improve the prognostic accuracy for an individual patient.

This review is aimed at those working with patients in neurocritical care, either in a clinical or research context. Firstly, some essential definitions are provided. Secondly, the anatomical and physiological basis for the release of biomarkers into the CSF is reviewed, including a synopsis of the function of the blood brain barrier (BBB). To illustrate the release of biomarkers from the cytosol of the damaged brain cells into the adjacent body fluid compartment, a video of an experimentally damaged neuron is shown. A list of potential biomarkers and those under current investigation is provided and the most important CSF biomarkers are discussed in detail. Finally, a summary of the evidence that CSF biomarkers improve prognostic accuracy is given, together with some recommendations for future biomarker research in neurocritical care.

2 Definitions

The definitions presented here were adapted and extended from the experience with biomarkers in oncology [9] and a recent workshop on biomarkers at the National Institutes of Health (NIH) on multiple sclerosis [10].

- Biomarker: "a characteristic that is objectively measured and evaluated as an indicator of normal biologic processes, pathogenic processes, or pharmacological responses to therapeutic intervention." [10] Biomarker may be sub–classified into:

  - Prognostic biomarkers: biomarkers which are associated with a clinical outcome, such as a time–to–event outcome [9].
  - Predictive biomarkers: biomarkers which can narrow the choices between treatment options [9].
  - Process biomarkers: biomarkers which allow monitoring of the dynamics and activity of pathological features.
  - Safety biomarkers: biomarkers which allow evaluation of the safety of treatments and give an early warning of unwanted side–effects.

- Surrogate endpoint: "defines a biomarker that is intended to serve as a substitute of a clinically meaningful endpoint and is expected to predict the effect of a therapeutic intervention or the evolution of disease. [10]"

- Clinical endpoint: "defines a meaningful measure which captures how a patient feels, functions or survives. [10]" Clinical endpoints may be sub–classified into:

  - Intermediate endpoint: represents "a clinical endpoint that is not the ultimate outcome but is nonetheless of real clinical usefulness. [10] " (e.g. the GCS or FOUR score)

  - Ultimate clinical outcome: represents "a clinical endpoint reflective of accumulation of irreversible morbidity and survival. [10]"

  - Time–to–event outcome: The time until a predefined event occurs, e.g. time to discontinuation of assisted ventilation, time to discharge from ITU or time to death.

In neurocritical care, where many patients are unresponsive or sedated, a biomarker provides additional information on damage to the brain parenchyma. It is my personal opinion that a CSF biomarker should always be regarded as an extension of the clinical assessment and judgement.

3 Blood–brain barrier

Any substances circulating in the bloodstream may be relatively harmless systemically, but could cause havoc by disturbing brain homoeostasis if they allowed into the ECF. For this reason the brain parenchyma is protected from the systemic circulation by the BBB. Some basic understanding of the BBB function is therefore necessary for the balanced interpretation of data on CSF biomarkers.

The blood–brain barrier (BBB) prevents the unselected diffusion of substances from the blood into the CSF (Figure 1 A). The blood–CSF barrier (BCB) in contrast is a filter which permits substances to diffuse from the blood into the CSF according to their molecular seize [11]. Strictly speaking one should separate between the BBB and BCB function, but for simplicity this is seldom done.

For assessment of the BBB/BCB function the current gold–standard is the measurement of albumin in the CSF (normal range 144–336 mg/L)[1] and serum (normal range 28.4–53.8 g/L) [12]. Because albumin is not produced intrathecally, all CSF albumin must be derived from the blood by diffusion through the meninges [13]. An intact BBB/BCB will only allow a small amount of albumin to diffuse into the CSF. The normal quotient of CSF to serum albumin calculates

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[1]Reference values are reflected by many pre-analytical and analytical factors. The reference values from one laboratory are not necessarily appropriate for another laboratory using different methods, or for another hospital providing a service to a different patient population.
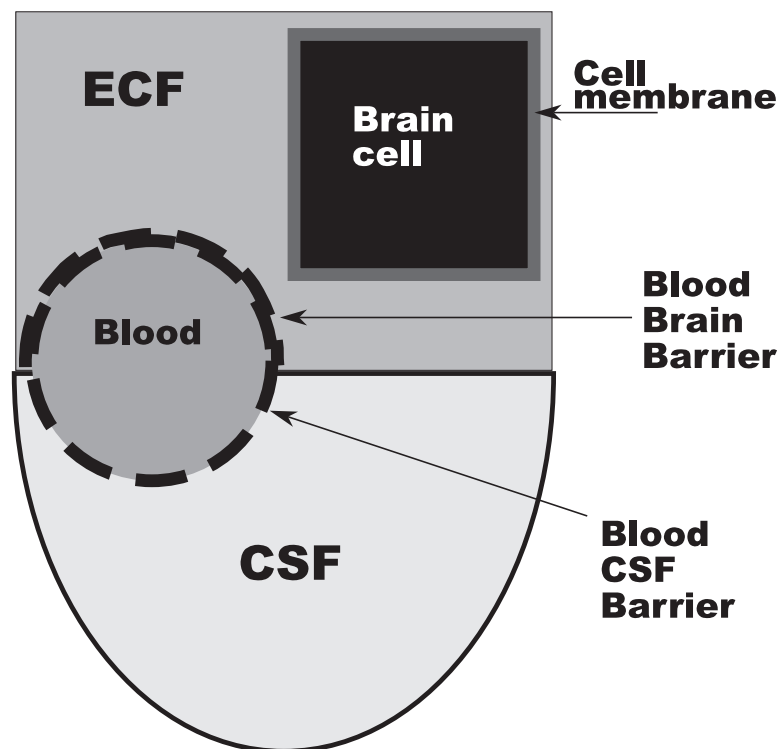
Figure 1: *(A) A simplified diagram of the tight blood–brain barrier (BBB) which separates the blood from the extracellular fluid (ECF) of the brain parenchyma. The very tight cell membrane prevents proteins from the cytosol diffusing into the ECF. The less tight blood–CSF barrier (BCB) allows substances to diffuse from the blood into the CSF.*
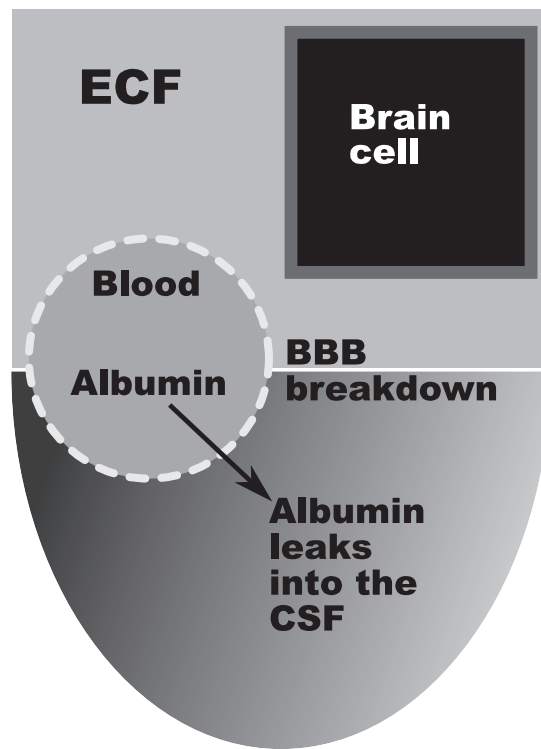
Figure 1: *(B) Breakdown of the blood–CSF barrier results in leakage of albumin from the blood into the CSF.*
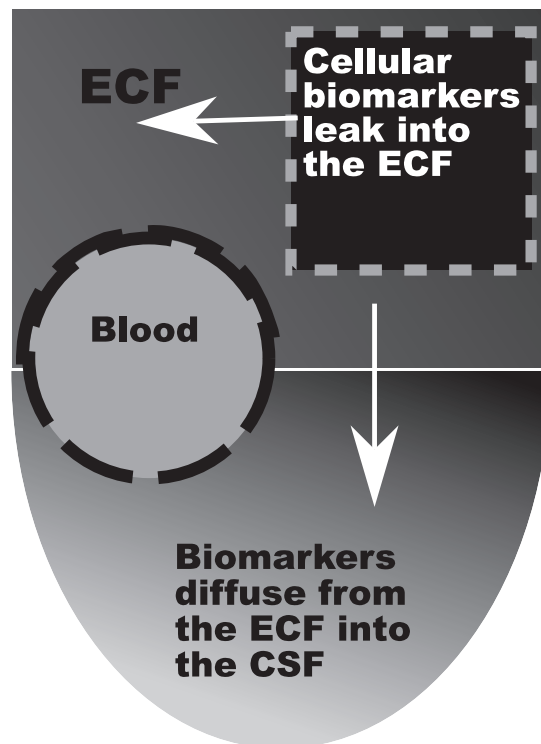
Figure 1: *(C) Cellular death following brain damage leads to disintegration of the cellular membrane. Biomarkers leak from the cytoplasm into the adjacent ECF. From the ECF these biomarkers then equilibrate with the CSF.*
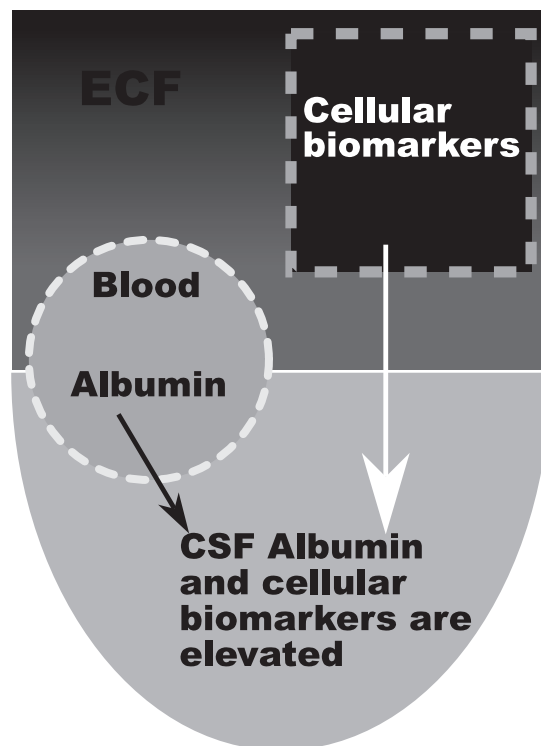
Figure 1: *(D) Illustrates the situation when both the blood-CSF barrier and the cell integrity break down. Because of the leakage of fluid and proteins from the blood into the CSF one would expect a dilution effect, artificially lowering the CSF levels of biomarkers released from dying brain cells.*

to 0.0018–0.0074 [12]. A breakdown of the BCB leads to leakage of serum albumin locally into the CSF (Figure 1 B) [2]. Consequently the CSF albumin rises and therefore the CSF to serum albumin quotient increases. The quotient of the CSF to serum albumin provides a widely accepted tool to approximate the integrity of the BBB. A CSF to serum albumin quotient > 0.0075 is abnormal[3]. Because the CSF is absorbed through the arachnoid villi (which have no barrier function) any substance present in the CSF will enter the blood. There is no known biomarker specific for the endothelial cells of the blood vessels in the brain. This is important to remember because the integrity of the BBB can only be tested by analysing both a blood and a CSF sample. Any conclusion about the integrity of the BBB based on an isolated blood sample is questionable.

Irreversible damage to the brain results from death, of a number of brain cells, either by necrosis or apoptosis causing the cellular membrane disintegrate (see video). This leads to leakage of biomarkers from the cytoplasm into the adjacent ECF (Figure 1 C). From the ECF these biomarkers diffuse into the CSF from where they can readily be sampled by lumbar puncture or via an extraventricular or a lumbar drain.

What happens if there is BBB breakdown and additional death of neurons? Figure 1 D illustrates how albumin and other proteins leaking from the blood into the CSF dilute the CSF concentration of proteins derived purely from the brain parenchyma. This is important to note, because high CSF levels of a biomarker such as neurofilaments [15] (or S100B [16]) in the context of BBB disruption, for example would still be regarded as a valid measure of damage to the CNS.

4 Cerebrospinal fluid

Normal CSF is clear and colourless. It consists of 99% of water and has a much lower protein concentration ($\approx$ 350 mg/L) [4] than the serum (70,000 mg/L).

The concentration of a CSF biomarker depends on:

1. If derived from the CNS parenchyma:

   - Intrathecal production
   - Leakage into the ECF
   - Diffusion into the CSF

2. If derived from the systemic circulation:

   - Serum concentration

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[2]Because there is currently no data on time matched ECF/CSF/blood albumin levels, the relationship of albumin levels to differential breakdown of the BBB and BCB is unknown.

[3]Thompson uses the "percentage albumin transfer" ($\frac{CSF_{Alb}}{Serum_{Alb}} * 100$ instead [14]. A percentage albumin transfer $>0.7\%$ is indicative of leakage of albumin from the serum through a damage BBB.)

[4]Please note that this value refers to the CSF *total* protein concentration which is higher then the CSF albumin concentration of 144–336 mg/L mentioned above.

- BBB integrity
- Hydrodynamic radius

About 70% of CSF is derived from the choroid plexus by filtration from the blood [17, 18]. The highest filtration rate ($\approx$ 40 mL per hour [19]) occurs naturally at night, in the recumbent position with increased hydrostatic pressure, which probably accounts for clinical symptoms such as headache and nausea suffered in the morning by patients with hydrocephalus. The remainder of the CSF comes from the ECF of the brain parenchyma and through the meninges and the blood–nerve barrier at level of the nerve roots. The CSF is modified on its passage from the ventricles to the lumbar sac and this is influenced by the CSF flow rate [20]. As a rule of thumb the concentration of proteins derived from the plasma is highest in the lumbar CSF and shows a rostro–caudal gradient, because of diffusion through the blood–nerve barrier along the length of the spinal cord.

Proteins derived from the brain parenchyma, called by some authors *brain–specific proteins* (BSP) or CNS–specific proteins, only make up about 10% of the total CSF protein content [11]. Concentrations of some of these BSPs are higher in the ventricular CSF and others higher in the lumbar CSF [13]. With the availability of microdialysis as a clinical and research tool it may be possible in future to obtain longitudinal (e.g. hourly) samples from the ECF and ventricular and lumbar CSF. Such samples would be extremely valuable for future CSF biomarker research.

5 CSF Biomarkers

Biomarkers can be put in to 6 broad categories, of which cell–type specific biomarkers are most relevant for prognosis in neurocritical care because they allow for a precise estimate of the amount of CNS tissue destruction.

1. CSF pigments

2. CSF metabolic biomarkers

3. CSF cell–type–specific biomarkers

4. CSF biomarkers for widespread CNS damage

5. CSF free–radicals and biomarkers of oxidative stress

6. CSF biomarkers for inflammatory/immunological processes

5.1 Pigments

The clear CSF changes colour with the addition of substances such as bilirubin (yellow) or haemoglobin (red) [21]. Spectrophotometry allows quantitative

measurement of pigments in the CSF [22, 23]. Because of the continuous conversion of haemoglobin to bilirubin, one expects oxyhaemoglobin levels to decrease as bilirubin levels increase following e.g. a subarachnoid haermorrhage (SAH). A secondary increase in CSF oxyhaemoglobin suggestive of micro–rebleeds, was observed in SAH [24]. Micro–rebleeds may be too small to be visible on CT, so the quantification of CSF pigments may allow investigation of the occurrence and prognostic implications of micro–rebleeds.

5.2 Metabolic biomarkers

These biomarkers represent the intermediate- and end–products of the metabolic pathways. A very small sample volume can rapidly be analysed. The basic metabolic pathways are shared by most cell types and are therefore good indicators of global impairment, but are not specific for any particular cell type or pathological process (see Table 1). CSF glucose and pyruvate are routinely measured. CSF lactate is also relevant in certain conditions. During ischaemia the cellular metabolism switches from the aerobic to the anaerobic pathway leading to an increase of lactate and decrease of pyruvate which indicates a lack of O_2 supply. Metabolic biomarkers were also amongst the first to be investigated in the extracellular fluid (ECF) using microdialysis [25].

5.3 Cell–type specific biomarkers

Cell–type specific biomarkers are proteins, lipids or circulating nucleic acids exclusively expressed by a specific cell–type, for example, neurofilaments are only expressed in neurons and their axons [15]. This is illustrated by a video on *the Journal's website*.

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              Link out to the video clip about here]
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Caption to the video clip to be shown on the Journal's website: the video shows a dorsal root ganglion with its axon in a culture dish located within a laser–dissection microscope. A laser beam is directed to the cell membrane of the neuron. As the membrane is cut open the content of the cytoplasm is released into the culture media. Next, the axon is severed by the laser beam. All the substances released into the culture medium can potentially be quantified and used as biomarkers.

In the brain, cell–type specific biomarkers are released into the extra–cellular fluid (ECF) following any form of cellular damage. From the ECF these biomarkers diffuse into the cerebrospinal fluid (CSF) and thence into the systemic circulation (Figure 1 C). Figure 2 illustrates how neurofilaments one(one example of a cell–type specific biomarker) are released.

Axonal degeneration

Nf

ECF → CSF

Blood

Amniotic fluid

Nf measurement:
**Nf levels allow
estimation of the degree
of axonal degeneration**
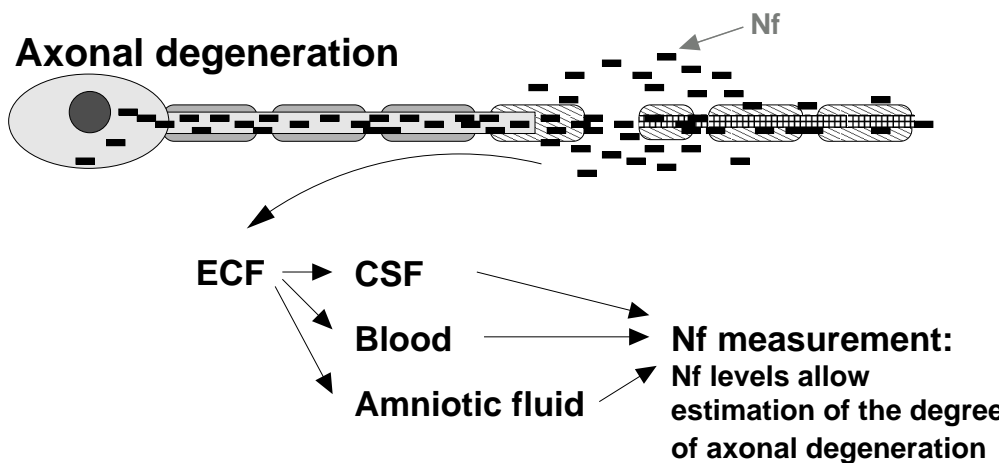
Figure 2: *Neurofilaments are released into the extracellular fluid (ECF) follow-ing axonal disintegration. From the ECF Nfs equilibrate with the adjacent body fluid compartment. Quantification of Nfs is therefore possible from the cere-brospinal fluid (CSF) [26, 27, 28], blood [29, 30] and amniotic fluid [31]. The degree of axonal degeneration is related to the amount of Nf measured in these body fluids. For this reason body fluid Nf levels permit estimation of the amount of axonal degeneration. Axonal degeneration is relevant because axonal loss is irreversible and a leading cause of persistent disability in an individual patient.*

A list of established and emerging CSF biomarkers is presented in Table 1. Only few are cell–type specific; most are indicative of more widespread damage to the CNS parenchyma.

Table 1: *Established and potential CSF biomarkers and their cellular sources for acute CNS damage, assuming a normal PNS.*

CSF Biomarker	Neuron & Axon	Astro-cyte	Micro-glia	Oligoden-drocyte	Choroid plexus	Blood
14-3-3γ	++	+	+	+		+
ABP	+					
AD7c-NTP	+					
Albumin						+
α spectrin	+	+	+	+	+	+
α(1)BG		+				
α–internexin	+					
ApoE			+			+
β–tubulin	+					
β-2-Microglobulin			+			
β-trace				(+)	+ [5]	
Clusterin	+	+		+	+	
Cystatin C	+				+	
EDG-8				+		
FABPs	+	+	+	+		+
FFA	+	+	+	+		+
Ferritin			+			+
GFAP		+				
Glucose	+	+	+	+		+
Glutamate	+	+	+	+		+
HK6					+	
HNE	+	+	+	+		+
Hypocretin–1	+ [6]					
Isoprostanes	+	+	+	+		+
Lactate	+	+	+	+		+
MAG				+		
MBP				+		
MDA	+	+	+	+		+
MOBP				+		
MOG				+		
NAA	++	+	+	+		
NCAM	+			+		
NOx	+	+	+			+
NSE	+					+
Neurotrophins	+	+	+	+		
Nf	++					
OMgp				+		
PLP				+		

[5] mainly derived from meningeal cells
[6] from hypothalamic neurons

PrPc	+				
Pyruvate	+	+	+	+	+
S100B		++		+	
SFas (sCD95)	+				
Tau	+	+	+	+	
Ubiquitin	+	+	+	+	+
Vimentin	+	+	+		

Neurofilaments Neurofilaments (Nf) are the key building blocks of the axonal cytoskeleton. The Nf protein is a heteropolymer composed of four subunits: a light (NfL), a medium (NfM), and a heavy (NfH) chain [32], and α–internexin [33, 34, 35]. Nf are almost exclusively expressed in neurons and axons [27, 15]. Following damage to the neuron and/or axon, the cytoplasmatic contents are released into the extracellular fluid (ECF) (Figure 2 and video). From the ECF Nf diffuse into other body fluid compartments including the cerebrospinal fluid (CSF) (Figure 1 C).

High–throughput analysis of Nfs is possible using enzyme–linked immune assays (ELISA). In-house ELISAs have been developed for NfL and NfH [26, 27, 28, 30]. These assays are robust and have been cross–validated [36, 37]. A commercial ELISA kit for quantification of the phosphorylated Nf heavy chain (pNfH) has recently become available (Chemicon, catalogue number NS170).

CSF Nf levels have been studied most extensively in patients suffering from a SAH, all studies revealing comparable results [27, 38, 36, 39, 40]. Following the bleed, CSF NfH and NfL levels are considerably elevated in all SAH patients when compared to controls. One longitudinal UK study suggested that there may be a secondary increase of CSF NfH levels [38]. This was confirmed in a subsequent longitudinal US study and interpreted as the consequence of secondary brain damage caused by delayed cerebral ischaemia. The study also demonstrated that patients with a poor outcome on the 6–month GOS (1 to 3) showed a secondary increase of ventricular CSF NfH levels about four days after the bleed [39]. Nylén *et al.* went on to show in a cross–sectional study that lumbar CSF NfL levels 10–14 days after the bleed were significantly higher in those patients with a bad recovery (GOSE 1 to 4) compared to those with a favourable outcome (GOSE 5 to 8) [40]. CSF NfH and NfL levels correlated with the initial severity of the injury [39, 40].

Norgren *et al.* showed that CSF NfL levels were elevated in 5/6 patients with stroke [28]. In a longitudinal study on 34 patients with a minor stroke, we found a weak correlation between CSF NfH levels and the modified Rankin score at discharge (unpublished data).

Rosén *et al.* showed that CSF NfL levels taken an average of 17.5 days after cardiac arrest predicted the one–year outcome on three scales: GOS, minimental state examination (MMSE) and activity of daily living (ADL) [41]. In this particular study the scoring on the GOS was inverse to convention [2, 42], thus explaining the positive correlation of the CSF NfL levels with the GOS (R=0.79) rather than the expected negative correlation [41].

Glial fibrillary acidic protein   Glial fibrillary acidic protein (GFAP) is a key building block of the cytoskeleton of the astrocyte. During astrocytosis this cytoskeleton rapidly reshapes and the tightly packed GFAP polymer loosens up within minutes, thus exposing more epitopes for recognition by anti–GFAP antibodies. This explains the extensive immunohistochemical staining with anti–GFAP antibodies of astrocytosis. Importantly, astrocytes are particularly vulnerable to ischaemia [43, 44, 45] and the measurement of GFAP released during astrocytosis and astroglial disintegration provides a tool for monitoring glial pathology.

A number of in–house GFAP ELISAs have been developed [46, 47, 48, 49, 50] and a commercial kit is available through Biovendor (Heidelberg, Germany, Cat No. RD 192072200).

Nylen *et al.* found that CSF GFAP levels were elevated in patients with vasculitis [51]. Inspection of Table 2 in reference [51] suggests that CSF GFAP levels are particularly high in patients with systemic lupus erythematosus (SLE) who also presented with focal neurology.

Gurnett *et al.* found levels of CSF GFAP (but not CSF NSE) to be increased in children following an epileptic seizure. CSF GFAP levels were highest in children with symptomatic epilepsy and prolonged seizures [52].

CSF levels of GFAP were found to be elevated in patients with SAH [53]. A subsequent longitudinal study showed CSF GFAP levels to be pathological in 93% of all samples [54]. By comparing non–survivors with survivors, significantly higher CSF GFAP levels were found for the former on day 2 and again on days 6 and 7 following the bleed. A simple model for the interpretation of CSF GFAP levels in patients with SAH was proposed based on these results (Figure 3). Given the number on studies on *serum* GFAP levels in patients with SAH and stroke [55, 55, 56, 57, 58] there is a lack of data on *CSF* GFAP.

Myelin proteins   Myelin proteins specific for the CNS and PNS consist of several hydrophobic proteins. *Myelin–associated glycoprotein* (MAG, $\approx$ 100kDa) is expressed both in the CNS and PNS and accounts for about 0.1-1% of total myelin protein [59]. Due to alternative mRNA splicing, two MAG isoforms (L–MAG, S–MAG) exist. *Myelin–associated oligodendrocytic protein* (MOBP) is a small protein present only in oligodendrocytes. *Myelin basic protein* (MBP) is expressed in the CNS and PNS in a variety of isoforms due to alternative splicing and an even larger number of post–translational modifications. *Myelin/oligodendrocyte glycoprotein* (MOG) is a transmembrane protein in the CNS. *Oligodendrocyte/myelin glycoprotein* (OMgp) is a 120 kDa glycosylated extracellular molecule in the CNS. The *proteolipid protein* belongs to the tetraspanins and is one of the most hydrophobic proteins known [59]. Of all these myelin proteins, it is mainly *myelin basic protein* (MBP) which has been measured in the CSF.

Myelin basic protein (MBP)   Myelin basic protein (MBP) is encoded on chromosome 18q23 and has 170 amino acid residues and a molecular weight of
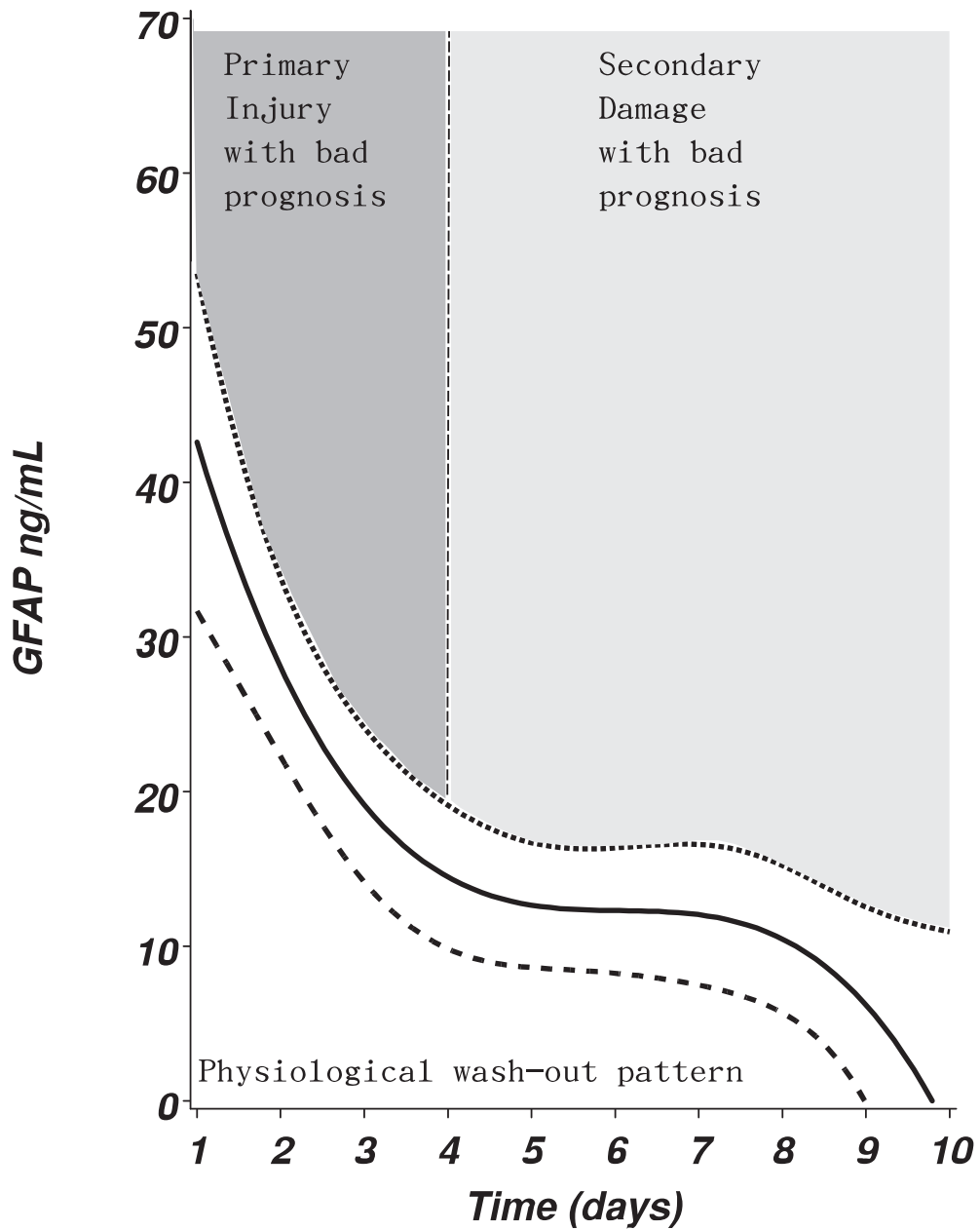
Figure 3: *A model how longitudinal CSF GFAP levels may help improving the prognostic accuracy in patients with SAH. CSF GFAP levels within the dark-grey area are suggestive of a bad prognosis based on the severity of the primary brain injury, whilst levels in the light–grey area suggest poor prognosis due to secondary brain damage. (Figure reprinted with permission from reference [53])*

18,500 Da. MBP is a major constituent of the myelin sheath of oligodendrocytes and Schwann cells. MBP–related transcripts are also present in the bone marrow and immune system, arising from "Golli–MBP" with 3 additional exons located upstream to the classical MBP.

Due to its basic properties the quantification of MBP is challenging. Numerous research groups have developed in–house methods and a range of commercial assays are available.

CSF MBP has been measured in patients with TBI [60, 61, 62, 63], hydrocephalus [64, 65, 66, 67], SAH [68], brain tumours and metastatic brain disease [69, 70, 71], asphyxia in children [72, 73], following chemotherapy [74, 75, 76, 77], stroke [62, 70, 78, 79, 80], encephalitis [70, 81, 82, 83, 80, 73], epilepsy [66], and following neurosurgery in patients with TBI and tumours [84]. MBP has probably been investigated most intensively in the CSF of patients suffering from multiple sclerosis (for review see reference [85]).

Noseworthy *et al.* were the first to describe a relationship between CSF MBP levels and outcome (GOS at 7 days, 3 months and 6 months) in patients with severe closed head injury [63]. In patients with SAH, CSF MBP levels were related to the occurrence of vasospasm–related delayed ischaemic neuronal deficit (DIND), infarct size and the 3 months GOS in one study [68]. In stroke CSF, MBP levels were related to the short–term outcome [78]. In asphyxiated full–term infants, increased CSF MBP levels were related to poor outcome (death or cerebral palsy at one year) [72]. In children with hydrocephalus and seizures, Levin *et al.* did not find CSF MBP levels to be of prognostic value [66].

Circulating nucleic acids Following cellular disintegration by apoptosis or necrosis, DNA and RNA are released into the blood [86]. Some cells may even to actively secrete DNA. Several methodological issues still need addressing, but oncology and neonatal medicine already use circulating nucleic acids for diagnostic and monitoring purposes [87]. Lam *et al.* investigated circulating nucleic acids in 44 patients suffering from stroke and found them to be a better predictor of outcome than S100B on the 6 months modified Rankin scale [88]. Circulating nucleic acids specific for different cell types could become an interesting future CSF biomarker.

5.4 CNS specific biomarkers

There are a number of CSF biomarkers in Table 1 which are not exclusively expressed by one single cell–type, but are nevertheless valuable in the assessment of damage to the CNS. Some of these may even be present in the serum due to systemic release, but if measured from the CSF they still allow for estimation of CNS damage.

Neuron specific enolase (NSE) Enolase is one of many glycolytic enzymes and consists of three subunits (α, β and γ) [89]. In the CNS the $\alpha\gamma$ and $\gamma\gamma$

isoforms are mainly localised within the neurons and therefore called neuron-specific enolase (NSE) [90]. Generally, NSE levels are now mainly used as a tumour marker for lung cancer [91].

Earlier studies on CSF NSE levels found them to be elevated in patients with acute encephalitis, meningitis, CVA, acute polyradiculoneuritis and brain tumours (see Figure 2 in reference [92]). This findings were later confirmed and the list extended to brain tumour and epilepsy [93]. This makes CSF NSE levels important in neurcritical care.

Some authors found CSF NSE to be increased in children and adults following an epileptic seizure, particularly after a prolonged seizure [94, 95, 96], but others did not [52, 97, 98]. Comparing CSF and serum NSE levels in a heterogeneous group of patients with meningitis, acute brain injury with impaired levels of consciousness, neurocysticerosis and normal controls, Lima *et al.* found the former to be related to the GOS at discharge or death [99]. In one study of patients with GBS, high CSF NSE values predicted a longer disease duration [100]. Following cardiac arrest, elevated CSF NSE levels found 72 hours after the event were related to functional outcome [101].

Pleines *et al.* showed CSF NSE levels to be considerably elevated in TBI patients for the first 3 days following the event and they slowly decreased over the next 2 weeks [102]. The mean CSF NSE levels correlated with the contusion size on CT, but did not correlate with outcome on the GOS [102]. Similarly Ross *et al.* did not find CSF NSE levels to correlate with the GOS, APACHE II or ISS [103].

CSF NSE levels were also markedly elevated in children with TBI compared to controls [104]. A secondary increase of CSF NSE levels was observed in 4 of 5 children with inflicted TBI (iTBI) (see Figure 2 in reference [104]). The authors note that iTBI in children is frequently associated with violent shaking and/or impact with a hard surface, in addition to delayed presentation to health care professionals, potentially leading to hypoxia and ischaemia. This leads to the suggestion that delayed neuronal death may be an important feature in iTBI [104]. This observation is consistent with an earlier study in asphyxiated infants which correlated elevated CSF NSE levels with outcome (death or cerebral palsy at 1 year) [72]. The Pittsburgh group then went on to investigate the prognostic value and found CSF NSE levels to correlate with the 6–months GOS [105]. However, their subgroup analysis showed that this was only the case in children over the age of four [105].

S100B Originally isolated from the bovine CNS by Moore in 1965 [106], the protein was named S100 because of its solubility in 100% saturated ammonium sulphate at neutral pH. S100B is now known to belong to a large family of S100 proteins [107]. S100B is coded on chromosome 21q22.3 [108] and consists of 91 amino acids and its molecular weight corresponds to 10–12 kDa. S100B is a water–soluble, cytoplasmic protein and contains 2 EF–hand calcium–binding domains (4 Ca^{2+} per dimer). The S100 proteins also bind Zn^{2+} and Cu^{2+} [109].

For quantification of S100B there are a number of in–house developed ELISAs [110, 92, 111, 112, 80] and a range of commercially available ELISAs such as the Can Ag S100 EIA (Can Ag Diagnostics AB, Gothenburg, Sweden), the ELISA NEXUS CX^{TM}S100 (Syn X Pharma INc., York, UK) and the Sangtec®100 IRMA, Liaison®Sangtec 100 and Sangtec 100 ELISA from DiaSorin AB (Bromma, Sweden) [113]. S100B levels are probably now most frequently used as a tumour marker for malignant melanoma [114, 115].

Earlier work on the CSF by Michetti *et al.* showed that S100B levels were elevated in patients with acute MS, acute encephalomyelitis, compression of the spinal cord and intracranial tumours [112]. The results were comparable to the observations by Sindic *et al.* who additionally found elevated CSF S100B levels in patients with stroke and SAH [111]. The spectrum of neurological diseases with elevated CSF S100B levels was broadened by Mokuno *et al.* to include patients with acute encephalitis, meningitis, SAH, CVA, multi–infarct dementia, Parkinson's disease, oral dyskinesias, cervical spondylosis, acute multiple sclerosis, acute and chronic polyradiculoneuritis and brain tumours (see Figure 2 in reference [92]). The findings in CVA were consistent with a later study [80]. Epilepsy has also been added to the list of acute neurological disorders with elevated CSF S100B levels [80, 116].

Over the last decade, a large number of studies has been published on serum S100B levels, mostly using the Sangtec®100 IRMA (for reviews see [117, 16, 118, 119]). Less data is available on CSF S100B levels which, as one study showed, did not correlate with those found in the serum [120]. The original work by Sindic *et al.* included a small number of patients with clinical follow–up data and longitudinal CSF S100B levels suggesting that in herpes encephalitis and transverse myelitis high CSF S100B levels may be a poor prognostic sign (Table 2 in reference [111]).

Pleines *et al.* showed that CSF S100B levels were considerably elevated in TBI patients for the first 3 days following the event and they then slowly decreased over the next 2 weeks [102]. CSF S100B but not CSF NSE levels correlated with the contusion lesion size on CT and the GOS. Kay *et al.* showed elevated CSF S100B levels in TBI, but there was no correlation with outcome data [121, 122]. Hayakata *et al.* showed CSF S100B levels to be elevated in severe TBI (GCS $<$ 8) and particularly in those with high ICPs [123]. Peak CSF S100B level were strongly correlated with the ICP. Importantly CSF S100B levels were higher in patients with unfavourable outcome compared to those with favourable outcome [123]. In children with iTBI CSF S100B levels correlated with the 6–months GOS [105].

High CSF S100B levels were described in patients with SAH [120]. In another study on SAH, CSF S100B levels on admission were also elevated but did not correlate with either injury severity (GCS) or outcome (GOS) [124]. Analysing the longitudinal profile of the same cohort, Kay *et al.* showed that the peak CSF S100B levels over a 7–day observation period correlated with the 3–month GOS [125].

Tau This microtubule-associated protein tau, also known as neurofibrillary tangle protein and paired helical filament-tau (PHF-tau). Tau is encoded on chromosome 17 and due to alternative splicing of the mRNA, six isoforms with a molecular weight of 48 to 68 kDa [126] exist in the human brain. Tau promotes microtubule stability and is used as a biomarker for neuro–axonal degeneration.

For quantification of tau most laboratories now use the hTau ELISA from Innogenetics (Ghent, Belgium).

Vandermeeren *et al.* were the first to report elevated CSF hTau levels in 5/11 patients with GBS [127]. Jin *et al.* then reported CSF hTau levels to be higher in patients with poor outcome (Hughe's functional grading scale score 2–5) compared to those with good outcome at 6 months [128]. Both studies contrasted with a small study by Süssmuth *et al.*, who found CSF hTau levels to be normal in GBS (n=5) [129]. All three groups used the same hTau ELISA from Innogenetics [127, 128, 129], suggesting that the different results may have been due to pre–analytical and clinical. Inspection of Figure 1 in reference [128] shows a bimodal distribution of CSF hTau levels for those patients with good outcome and it would be interesting to know from all the studies whether those patients with low CSF hTau levels had predominantly demyelinating pathology on electrophsysiological studies. Additionally, distal versus proximal axonal damage may account for the discrepancy [130]. Ost *et al.* found CSF tau levels to correlate with the 1–year outcome following TBI on the GOS, NIHSS and the Barthel daily living index [131].

Zemlan *et al.* developed antibodies directed against 30 to 50 kDa cleaved tau (c-tau) [132]. They showed that the antibodies (ctau7, cTau8, cTau12) bind to the interior portion (Pro^{251}–MET^{419}) of the tau sequence with the N- and C–terminal amino acids being cleaved. The highest CSF tau levels were observed in patients with TBI when compared to MS, NPH or non–neurological controls [132]. Subsequently the authors measured CSF c-tau in patients with TBI, neurological- and non–neurological controls [133]. Initial c-tau levels were about 40,000-fold higher in the CSF of patients with TBI compared to either control group. C-tau and the GCS were independent predictors of the clinical outcome (GOS on discharge). The sensitivity/specificity limits for predicting an unfavourable outcome were 92%/94% for c-tau and 50%/100% for the GCS [133]. Quantification of c-tau opens an interesting future possiblity for CSF biomarker studies.

Amyloid beta peptide (ABP) The $\approx$ 700 amino acid large amyloid precursor protein (APP) is cleaved at the β and γ sites by secretases into amyloidβ sequences of 40/43 residues (ABP). The numbers behind the ABP refer to the cleavage site of the protein fragment (e.g. ABP 1-42). The amyloid beta peptide (ABP) is important in the pathogenesis of Alzheimer's disease. Nicoll *et al.* suggested a link between ABP deposition in the brain of patients with head injury and presence of the APOE epsilon4 allele [134]. In the CSF decreased levels of ABP 1-42 provide valuable information for the differential diagnosis of neu-

rodegenerative dementias [135]. CSF ABP has also been measured in patients with ischaemic stroke [136], SAH [125, 124], TBI [121, 122, 137, 138, 139] and acute bacterial meningitis [140].

In SAH both the CSF ABP 1-40 and 1-42 fraction were lower compared with CSF ABP levels in controls [125, 124]. The maximum decrease in the CSF ABP concentration correlated with the 3–months outcome in a longitudinal study [124].

In TBI, Kay *et al.* described the CSF ABP 1-40 and 1-42 as remaining low for at least 5 days following the injury [122]. There was no relationship with the GOS. In contrast, Franz *et al.* who found CSF ABP 1-42 levels to distinguish between good (GOS 4-5) and bad (GOS 1-3) outcome with a sensitivity of 100% and a specificity of 82% [138]. In a subsequent scientific exchange, Blennow and Nellgård make the important point that CSF ABP levels are about 5–fold higher in lumbar compared to ventricular CSF in patients with Alzheimer's disease [141]. In their reply, Franz *et al.* confirmed this point for their TBI cohort, showing about 2–fold higher ABP 1-42 levels in the lumbar compared to the ventricular CSF though the latter group had a worse outcome, potentially introducing a sample bias. All three studies [122, 138, 141] contrast with an earlier report on showing an increase of CSF ABP 1-42 levels in TBI patients [142].

Apolipoprotein E   Polymorphism of the apolipoproteins E gene (APOE) influences the risk of the development of the sporadic from of Alzheimer's disease (AD) and is a risk factor for more severe disease in almost every neurological disorder except GBS [143, 144, 145]. In patients suffering from a head injury, Teasdale *et al.* was first to show that the presence of the APOE ϵ4 allele was associated with a significantly higher risk of mortality [146].

In the CNS ApoE is relevant for transport of cholesterol and lipids [147]. In TBI a decrease in CSF apoE levels was observed [121, 122], but CSF apoE levels did not correlate with the GOS [122]. Following SAH CSF apoE levels were also reduced, albeit to a lesser degree than in TBI [124, 125], and correlated with the 3–months GOS [125]. In ischaemic stroke CSF apoE levels were no different from the control group [136].

Hypocretin-1   Hypocretin-1 (synonymous orexin-A) is a neurotransmitter released from the posterior hypothalamus, and is relevant to the modulation of various different psychological functions [148, 149]. Since Lin *et al.* recognised that CSF hypocretin-1 levels were decreased in the CSF of patients with narcolepsy, this small peptide has been studied in a large range of neurological conditions (for a review see [150]). Rejdak *et al.* showed in a longitudinal study that CSF hypocretin-1 levels were decreased in patients with acute brain injury (TBI and SAH) and remained undetectable in two patients with a destructive lesion of the thalamus/midbrain [151]. Because excessive fatigue or daytime sleepiness has been reported following a SAH [152], the authors suggested that a deficient hypocretin/orexin system may in part have been responsible for abnormalities in the sleep–wake cycle following acute brain injury. The findings

were consistent with those of a larger cross–sectional study on patients suffering from TBI [153]. Another small longitudinal study on SAH patients (n=15) confirmed that CSF hypocretin-1 levels decreased after SAH and were undetectable in those with a GCS of less than 8 on admission [154]. This was in line with a further study on 15 patients with SAH, with a secondary decrease and particular low CSF hypocretin-1 levels in those who developed delayed ischaemic neuronal deficit (DIND) [155]. In 4/6 patients suffering from a paraneoplastic encephalitis (positive for anti–MA antibodies) and one patient with Hashimoto's encephalopathy, excessive daytime sleepiness was also associated with undetectable CSF hypocretin-1 levels [156, 157]. Remarkably, the diurnal variation of CSF hypocretin-1 levels (about 4%) has been described in healthy controls, suggesting that sampling time is of minor importance [158].

Alpha spectrin   Two independent studies demonstrated that CSF Alpha-II spectrin levels were elevated in patients with severe TBI (GCS<8) [159, 160]. The longitudinal data analysis of the study by Cardali and Maugeri showed that those patients who maintained high CSF levels of alpha-II spectrin and spectrin breakdown products (SBDPs) had a poorer outcome on the 6–months GOS [159]. In an experimental rat ischaemia model CSF α-spectrin cleavage products were also detectable [161]. These authors point out the widespread distribution of α-spectrin in essentially all non–neuronal cells [162] makes it unlikely that CSF α-spectrin will ever become a specific biomarker for brain injury [161]. Nevertheless Pineda *et al.* demonstrated a differential time profile for calpain and caspase-3 mediated SBDPs [163]. In their study SBDPs were related to the 6 months GOS.

Cytochrome C   Cytochrome C has been used as a biomarker for apoptosis. Statchell *et al.* showed CSF cytochrome C levels to be increased in children with iTBI compared to controls [164].

Taurine   The amino acid taurine is currently best known as an additive to energising drinks. Experimentally taurine was released during cellular oedema, e.g. from astrocytes, and possessed anti–oxidant properties. Seki *et al.* demonstrated that CSF taurine levels were marginally increased in the CSF of patients with TBI when compared to patients with normal pressure hydrocephalus [165]. In an experimental TBI model in rats, Stover *et al.* showed that CSF taurine levels were increased about 8 hours after the injury [166]. The same group described an increase of CSF taurine levels in patients with subdural or epidural haematomas, contusions and generalised brain oedema [167].

NT-proBNP   N-terminal (NT)-pro brain natriuretic peptide BNP) was shown to be elevated in a longitudinal study on ventricular CSF from TBI patients, but CSF NG-proBNP levels were not correlated with the 3–months GOS [168].

Ubiquitin Majetschak *et al.* showed that CSF ubiquitin levels continued to rise until death in 3 TBI patients, but recovered in 3 survivors from TBI [169]. The substantiated their findings by an experimental TBi study in pigs, showing that the biological half life of ubiquitin was about 1.3 hours. Contamination of the CSF by erythrocytes and consequent lysis accounted for about 15% of the CSF ubiquitin levels [169].

14-3-3 The 14-3-3 proteins ($\approx$ 30 kDa) are highly homologous, dimeric proteins, expressed in almost all eukaryotic cells from yeast to humans [170]. In humans one separate gene encodes for each of the seven isoforms (β, γ, ϵ, η, σ, τ, ζ) [171]. Five major isoforms are found in the CNS, named α to η. 14-3-3β is phosphorylated to 14-3-3α, and 14-3-3ζ is phosphorylated to 14-3-3δ. 14-3-3 proteins are involved in the regulation of the cell–cycle, cell signalling, intracellular trafficking and shaping of the cytoskeleton [170, 172, 173]. Since Boston *et al.* first described the presence of 14-3-3 in the CSF [174], the 14-3-3γ isoform has been most commonly used as a CSF biomarker. 14-3-3γ is expressed in neurons, astrocytes, oligodendrocytes, and microglia [175]. To the best of my knowledge attempts to develop an ELISA for quantification of 14-3-3 isoforms has so far not been successful. This may be due to the epitopes of the different isoforms being cryptogenic, and/or the difficulties in raising antibodies against such ubiquitous proteins. Most laboratories currently use immunoblotting techniques for detection of 14-3-3γ as described elsewhere [176].

CSF 14-3-3γ is most frequently used as a biomarker in the differential diagnosis of Creuzfeld–Jakob disease [177] but, as with all other proteins 14-3-3γ is essentially released following cellular damage. CSF 14-3-3γ has been reported in patients with GBS [178], Hashimoto's encephalopathy [179, 180], meningitis and encephalitis [181, 175], stroke–like episodes [175] and transverse myelitis [182].

Nestin Nestin belongs to the intermediate filaments and is found in CNS progenitor cells. One group found CSF nestin levels to be decreased following asphyxia [183].

Albumin Ischaemia–modified albumin has been found in the serum in patients with stroke [184, 185, 186]. It can also be detected virtually whenever a small amount of hypoxia (e.g. anaerobic metabolism) occurs, including physical exercise [187, 188]. Ischaemia–modified albumin has not yet been investigated in the CSF. As discussed further down the timing of sampling is important for interpretation of the data.

5.5 Oxidative stress and free radicals

Oxidative stress occurs when the physiological balance between oxidants and antioxidants is shifted towards the former. The production of oxidants such as free radicals is increased in patients with ischaemic brain damage [189]. This increase of free radicals causes oxidative stress which compounds existing brain damage. Therefore biomarkers of oxidative stress have the potential to allow monitoring of secondary brain injury and may be relevant for prognosis. Following ischaemia some Ca^{2+}–activated enzymes such as cyclo–oxygenase (COX) and phospholipase A_2 produce oxygen free radicals, whilst others such as nitric oxide synthase (nNOS and iNOS) produce nitric oxide (NO). Iron is another important source of free radicals (OH . via the Fenton reaction), particularly after haemorrhagic brain injury.

Nitric oxide (NO) The methodological challenge is that free radicals cannot readily be measured *in vivo*. The biological half–life of NO is less then 5 seconds, therefore downstream metabolites need to be quantified instead. For example, the NO metabolites NO_2 and NO_3 (commonly denoted as NOx for NO metabolites) can be measured in the CSF using a high–throughput technique [190, 191]. CSF NOx levels did distinguish survivors from non–survivors in one study [38]. For a comprehensive review on NO, the reader is referred to more substantial reviews [192, 193, 194].

Excitatory amino acids Excitatory acids such as glutamate are neurotoxic if released in excess. There is an excellent review by Meldrum and Garthwaite on glutamate exitotoxicity [195].

Palmer *et al.* found that CSF glutamate levels increased for 3 days following TBI. CSF glutamate levels were about 7 μM, which was considered to be sufficiently high to cause further excitotoxicity [196]. Elevated CSF glutamate concentrations were also found by Baker *et al.* in a separate longitudinal study on TBI. The results were consistent with those by Zhang *et al.* who found CSF glutamate (and aspartate) levels to be elevated in TBI compared to neurological control patients and to correlate with the 3–months GOS [197]. Another longitudinal study found that the highest CSF glutamate levels peaked about 48 hours after TBI [198]. The same group then went on to describe an increase of CSF glutamate levels in patients with subdural or epidural haematomas, contusions and generalised brain oedema [167]. CSF glutamate levels were also increased in children with TBI [199]. These findings are in line with those obtained for CSF glutamate in an experimental study on TBI in rats [166].

Lipid peroxidation For lipid peroxidation, the unstable aldehydes malondialdehyde (MDA) and 4-hydroxynonenal (HNE) have been measured alongside thiobarbituric acid-reactive substances (TBARs). MDA has been related with stroke size and outcome [200].

Free fatty acids (FFA)   Elevated levels of FFAs have been found in the CSF of patients with stroke, TBI, SAH. CSF FFAs levels were related to injury severity and outcome (for review see reference [201]).

Isoprostanes   Isoprostanes are non–enzymatically synthesised from polyunsaturated fatty acids such as e.g. arachidonic acid [202]. They are released in their free form by phospholipases [203]. Importantly isoprostanes are relatively stable [203, 204]. F_2-isoprostanes appear to be more reliable biomarkers for lipid peroxidation than e.g. MDA. Importantly, they are not only a metabolic product, but also have important biological functions [203]. For example the 15-F_{2t}-IsoP is an important vasoconstrictor, alongside 9-epi-15-F_{2t}-IsoP and 15-epi-15-F_{2t}-IsoP, with the E–series probably being even more potent vasoconstrictors (e.g. 15-E_{2t}-IsoP) [205]. CSF levels of F2–isoprostanes have recently been measured in patients with SAH [206]. Peak and mean CSF F_2-isoprostane levels correlated strongly with poor outcome, although these correlations were less impressive for plasma F2–isoprostane levels [206]. In patients with severe TBI (GCS<8) CSF F2-isoprostane levels peaked on day one after the injury and were significantly higher in males than females, suggesting that the degree of lipid peroxidation differs with gender in TBI [207]. In children suffering from severe TBI, CSF F2-isoprostane levels were higher compared to control CSF when [208]. Wagner *et al.* showed in a longitudinal study that CSF F2-isoprostane levels on day one correlated inversely with the 6–months GOS in women suffering from TBI [209].

Fatty acid binding proteins (FABPs)   These are $\approx$ 15 kDa proteins important for the uptake, transport and metabolism of fatty acids. Initially isolated from the heart muscle (H-FABP), they were subsequently found in many other cells including the brain [210]. In fact, FABs are present in all cells utilising fatty acids. Because they are not cell–type specific, and because their small molecular size allows for easy passage into the CSF, elevated CSF levels may be artefactual due to systemic involvement [211]. However, two studies independently found serum FABPs to be better prognostic indicators than serum S100B in patients with stroke [212, 213].

5.6   Inflammatory and immunological biomarker

The brain is an immunoprivileged organ. Inflammatory processes in brain tissue are tightly controlled [214]. The spectrum of CSF biomarkers relevant to the inflammatory and immunological diseases of the CNS is far beyond the scope of this review. Widening the spectrum from neurocritical care to the general intensive care unit, such biomarkers may also help to identify those patients who may be at risk of developing secondary brain damage due to critical illness [215, 216, 217]. Most inflammatory and immunological CSF biomarkers are, according to the introductory definition, "process biomarkers".

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6   The future of CSF biomarkers

Proteomics   2D gel electrophoresis is a well established technique that allows for separation of proteins according to molecular mass and charge. With the advent of mass spectroscopy, 2D gel electrophoresis has been re-employed to identify potential new biomarkers. Conti *et al.* found the acute phase proteins alpha-1 antitrypsin, haptoglobin 1 alpha1, alpha2 and beta to be increased in the CSF following TBI [218]. Interestingly the carboxyl–terminal portion of fibrinogen beta was only elevated in the CSF of patients with TBI, suggesting increased fibrinolysis in these patients [218]. Gao *et al.* have recently confirmed the results by Conti *et al.* Acute phase proteins and haptoglobins were increased in the CSF of patients with nTBI [219]. The authors also found CSF levels of prostaglandin–D(2)–synthetase and cystatin C to be higher in patients with iTBI when compared to patients with nTBI [219].

Burgess *et al.* performed a comparative study on ante- and postmortem CSF in in comparison to CSF in order to identify putative new CSF candidate biomarkers indicative for brain cell death [220]. These authors compiled an impressive list of 299 potential CSF biomarkers [220]. In a cell–culture model Siman *et al.* described release of over 60 cytoskeletal proteins from neurons [161].

7   Pitfalls

A range of pre–analytical and analytical pitfalls deserve to be mentioned. The CSF sampling technique, transport, time to storage and storage conditions should be standardised. Ideally one would like to have three serial tubes of CSF with at least 3 mL collected in a polypropylene tube. The reason for this is that most proteins are charged negatively and would bind to the positively charged surface of polystyrene or glass tubes, potentially leading to artificially low CSF levels of the biomarker in question. Samples should be spun down and the supernatant stored at least at -20°C within one hour of collection. Longer transport and sample handling times may cause degradation of the biomarker and cause artificial results. Needless to say, sample storage in multiple aliquots of a small volume (e.g. 500 μL to 1 mL in e.g. 1.5 mL Eppendorf tubes) allows for easy future analysis of several biomarkers, including the sending out of samples to other laboratories and avoiding repeated freeze–thaw cycles.

There are some simple analytical facts which help to guide the analysis of data. For example, the intra–assay coefficient of variation (CV = $\frac{SD}{mean} * 100$) of a technique indicates the degree of variation which may occur between performance of the same assay on the same sample at different time–points. For routine laboratory work a CV of less than 10% is desirable. Many assays developed for research are still in their infancy and will not achieve this target. This may not be critical as long as one remembers that an assay with a CV of, say, 20% cannot reliably be used to detect a difference between two groups (or

two subsequent samples) of, say, 4%. As a rule of thumb, the CV of a method determines how much trust one puts into the statistical result on group differences. In order to make publications comparable it is desirable to have some basic information about the methods such as the detection limit, sensitivity, linear range, parallelism and stability of the biomarker. For example it should come as no surprise if levels of a biomarker that is stable for one month at 4°C are higher in samples from a study group, compared to "historical samples" from a control group collected 5 years earlier. Well–defined upper reference values determined in a large reference cohort are helpful for future comparison as they act as a benchmark. All analysis should be performed with the analyst being blinded to all other information.

Most patients in neurocritical care will receive large quantities of intravascular fluids. Levesl of many biomarkers in the blood may be reduced simply because of an i.v. fluid–related dilution effect. In situations like this one can use an addiotional measure, e.g. the haemotocrit as guidance for data analysis.

Substantial suggestions for future biomarker studies have been made by McShane *et al.* (see Table 1 in reference [9]) and offer themselves for adaption to other medical subspecialties.

8   Conclusion

Table 2 summarises the CSF biomarkers which were related to outcome data and may therefore be of value for improving the prognostic accuracy in acute CNS disease. This list is small compared to Table 1 and the inclusion of α–spectrin may be debatable [161]. Biomarker stability, accuracy of the analytical technique employed, study design and the pitfalls discussed above all take their toll. Two studies compared the prognostic accuracy of a CSF biomarker with the GCS [1, 2] in TBI [133] or established neurophysiological criteria [221] in GBS [130]. Both found CSF biomarkers to give the most accurate prognosis [133, 130].

The challenge for future studies is to combine CSF biomarkers with solid outcome data.

Table 2: *Summary table for those CSF biomarkers which were related to clinical outcome scales and may be of help in improving the prognostic accuracy in acute CNS disorders.*

CSF biomarker	Disease	Outcome measure	Reference
α-spectrin [7]	TBI	GOS	[159, 163]
ABP 1-42	SAH	GOS	[124]
ABP 1-42	TBI	GOS	[138] [8]
ApoE	SAH	GOS	[125]
Circulating nucleic acids	stroke	modified Rankin	[88]
F2-isoprostanes	SAH	GOS	[206]
F2-isoprostanes	TBI	GOS	[209]
FFA	TBI, SAH, stroke	GOS	[201]
GFAP	SAH	Survival	[54]
Glutamate	TBI	GOS	[197]
MBP	SAH	GOS	[68]
MBP	Stroke	short term prognosis	[78]
MBP	TBI	GOS	[63]
NOx	TBI & SAH	Survival	[38]
NSE	GBS	disease duration	[100]
NSE	cardiac arrest	functional outcome	[101]
NSE	iTBI	Survival, GOS	[72, 105] [9]
NfH	GBS	F–score, MRCS	[130]
NfH & NfL	SAH	GOS	[39, 40]
NfL	Cardiac arrest	GOS	[41]
S100B	SAH	GOS	[125]
S100B	TBI	GOS	[102, 123] [10]
S100B	iTBI	GOS	[105]
Tau	GBS	F–score	[128]
Tau	TBI	GOS	[133, 131]

9 Acknowledgements

I apologise to all colleagues whose work has not been cited due to space limitations.

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[7] α-spectrin is also expressed in non–neuronal cells [162].

[8] These results are not consistent with [122]

[9] No such correlations were found in studies on TBI in adults [102, 103].

[10] These results were not consistent with [121, 122].

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