Biomarkers of primary and evolving damage in traumatic and ischemic brain injury: diagnosis, prognosis, probing mechanisms, and therapeutic decision making

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Purpose of review

Emerging data suggest that biomarkers of brain injury have potential utility as diagnostic, prognostic, and therapeutic adjuncts in the setting of traumatic and ischemic brain injury. Two approaches are being used, namely, assessing markers of structural damage and quantifying mediators of the cellular, biochemical, or molecular cascades in secondary injury or repair. Novel proteomic, multiplex, and lipidomic methods are also being applied.

Recent findings

Biochemical markers of neuronal, glial, and axonal damage such as neuron-specific enolase, S100B, and myelin basic protein, respectively, are readily detectable in biological samples such as serum or cerebrospinal fluid and are being studied in patients with ischemic and traumatic brain injury. In addition, a number of studies have demonstrated that novel tools to assess simultaneously multiple biomarkers can provide unique insight such as details on specific molecular participants in cell death cascades, inflammation, or oxidative stress.

Summary

Multifaceted cellular, biochemical, and molecular monitoring of proteins and lipids is logical as an adjunct to guiding therapies and improving outcomes in traumatic and ischemic brain injury and we appear to be on the verge of a breakthrough with the use of these markers as diagnostic, prognostic, and monitoring adjuncts, in neurointensive care.

Keywords

cardiac arrest, multiplex, proteomics, stroke, traumatic brain injury lipidomics

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Introduction

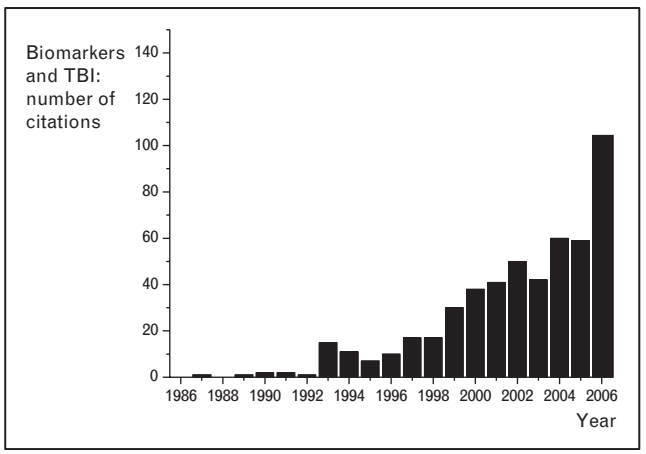
The topic of biomarkers in traumatic and ischemic brain injury is a vast one that is challenging to address comprehensively in a focused review. We will concentrate on key studies in clinical material where possible. Only a glimpse of this extremely timely topic can be provided. We believe that it is more important to focus on protein and lipid markers rather than genomic markers. Given that insults such as traumatic and ischemic brain injury are routinely accompanied by either regional or global energy failure, and associated with disturbed protein synthesis, gene array and other techniques cannot be counted upon to provide reliable information regarding protein expression. Indeed, the inhibition of protein synthesis is used as a definition of the ischemic penumbra in stroke [1]. Similarly, posttranslational modification of proteins is a critical aspect of the injury response and is not assessed with genomic approaches. Our group has a long-standing interest in biomarker research and this review will summarize some of our work while also integrating in a number of important studies in the field. Investigators have invariably taken advantage of a number of clinical resources, including, cerebrospinal fluid (CSF), serum, and brain interstitial fluid collected during cerebral microdialysis. Occasionally, clinical studies have also corroborated the presence of a mechanism or marker quantified in CSF with molecular work in human cerebral contusions resected from patients with severe injury and life-threatening cerebral edema [2–4]. By and large, there has been fidelity across samples. CSF and microdialysis have been used predominantly for mechanistic work, while more traditional studies assessing biomarkers for clinical use have appropriately targeted the use of serum, which would be readily available in all patients, with a range of injury severity and disease processes. CSF

is much more available in patients with severe traumatic brain injury (TBI), in light of the use of CSF drainage in the management of intracranial hypertension. As such, the clinical TBI literature is substantially richer with CSF biomarker studies than that seen for either stroke or cardiac arrest. Given the fact that microdialysis is still largely a research tool, whereas either serum or CSF is more generally available in patients with central nervous system (CNS) injury, we will focus this review on studies of biomarkers in CSF and serum. Finally, there have been a host of reviews and primary citations on this topic that have discussed the theoretical potential of this emerging field. Many of those reports, however, lack data. In this review, we will highlight reports that present data from either experimental models or patients in the setting of acute brain injury.

Historical perspective on biomarkers of brain injury

Searching with the terms 'biomarker' and 'traumatic brain injury (TBI)' on *PubMed* provided 516 references. Remarkably, nearly 90% of those citations were published in the last 10 years (Fig. 1). Much of the apparent surge in investigation of this topic, however, relates simply to the recent use or broadening the definition of the term 'biomarker'. There are two approaches to assessing 'biomarkers' of brain injury: directly surveying structural damage using a specific unique marker (or markers) of tissue damage, and measuring aspects of the cellular, biochemical, or molecular cascades in the secondary injury (or repair) response.

Figure 1 Plot of the estimated number of citations on *PubMed* versus year



The graph demonstrates the remarkable recent surge in interest in the topic of biomarkers of brain injury. Some of the apparent increase is related to the recent popularity of the term 'biomarker' since markers of brain injury have been suggested as potential clinical diagnostic or prognostic tools for nearly 20 years [19,20]. TBI, traumatic brain injury.

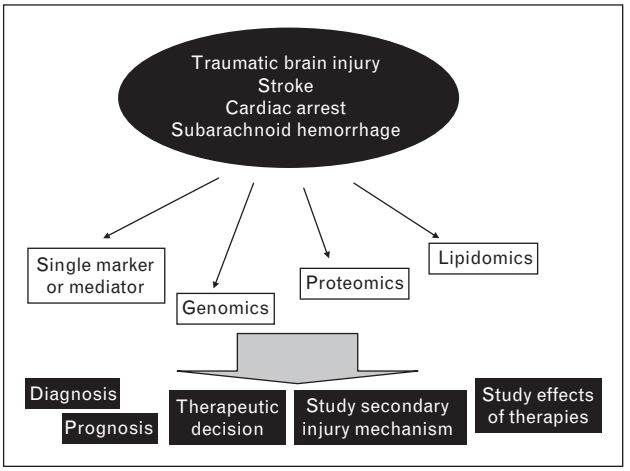
In the field of TBI, two early studies of 'biomarkers' and brain injury are noteworthy, and both were carried out by assessing ventricular CSF in patients with severe TBI. Over 30 years ago, Rudman *et al.* [5] published a classic study on CSF cyclic AMP (cAMP) levels in TBI, as a putative marker of the depth of coma after injury. cAMP levels were low early after injury and progressively increased as Glasgow Coma Scale (GCS) score improved, presumably related to increases in synaptic activity with resultant increases in second messenger production. A second series of seminal reports in the field of biomarkers in brain injury is the work of McClain *et al.* [6,7], who published the first report on CSF cytokines in human TBI and described increases in IL-1 and IL-6 as part of the tissue injury response. These and other early studies launched a sea of reports on the evolution of the mediators involved in the secondary tissue injury response to TBI, such as cytokines, growth factors, 'danger signals', and other effector molecules in severe TBI [8–18].

In the field of cerebral ischemia, the seminal work of Vaagenes *et al.* [19] in experimental cardiac arrest in dog models of ventricular fibrillation used an alternative approach, namely assessing the time course of accumulation and the concentration of a biomarker of structural brain damage, namely CSF levels of the brain isoform of creatine phosphokinase (CPKbb). Initial studies in a canine model of cardiac arrest were later translated to patient care [20]. Remarkably, despite the routine use of serum CPK as a 'biomarker' of myocardial injury, the use of CPKbb levels did not catch on in neurointensive care. One possible reason is the lack of effective therapies available for treatment. These two approaches, assessing markers of structural damage versus quantifying mediators of the cellular, biochemical, or molecular cascades in the secondary injury (or repair) response will serve as the framework for the additional studies discussed in this review. In addition, these different classes of biomarkers have potential to be useful in many ways including serving as diagnostic adjuncts, aiding in prognostication, influencing therapeutic decisions, studying key mechanisms in the injury cascade, and studying the effects of therapies (Fig. 2).

Biomarkers of structural damage

There are many structural markers of brain injury that have been examined in both TBI and brain ischemia. Logically, biomarkers reflecting damage and release from each of the major cell types/structures in brain parenchyma have been studied, namely, astrocytes, neurons, and axons. One marker that has received considerable attention in CNS injury has been S100B, a calcium binding protein localized predominantly in astroglia [21–23]. S100B has shown effectiveness as a diagnostic marker

Figure 2 Potential use of biomarkers in neurointensive care



in the setting of TBI, where it has been shown to be sensitive or specific, and in acute stroke, cardiac arrest, and subarachnoid hemorrhage [24••,25–27]. An advantage of S100B is the fact that it seems to be effective as a diagnostic marker of CNS injury, and some associated complications, such as hemorrhagic transformation and vasospasm, when assessed in either CSF or serum [24••,25–30]. One of the limitations to the use of S100B as a potential screening agent for brain injury in neurointensive care is the lack of specificity in the setting of hemorrhagic shock or circulatory arrest, such as in cardiopulmonary bypass [31,32]. Extracerebral sources appear to produce increases in serum levels in these settings. Similarly, its short half-life is a limitation, although S100B can be monitored in a delayed fashion in urine [33]. Whether urinary concentrations can be used to quantify the extent of damage, however, remains unclear. Finally, S100B is not a useful marker in children less than 2 years of age due to high normative values in that age group [34,35]. S100B has been reported to serve as an early warning monitor of evolving devastating brain injury, since Pelinka *et al.* [21] reported that delayed increases predict death in patients with severe TBI. Serum S100B is, thus, a logical marker to consider for inclusion in a panel of brain biomarkers for clinical use in neurointensive care, similar to what is currently used in assessment of hepatic injury.

Neuron specific enolase (NSE) is a glycolytic enzyme localized predominantly in neuronal cytoplasm. NSE, like S100B, has similarly shown sensitivity or specificity in TBI, stroke, and cardiac arrest [24••,27,28,36–43]. NSE does not appear to exhibit the age-dependent liabilities seen with S100B in the pediatric age group [28,35]. Similar to S100B, however, there have been reports of false positive values in the setting of combined CNS injury plus shock [44,45]. A second limitation of NSE is the occurrence of false positive values in the setting of hemolysis [25]. Nevertheless, like S100B, NSE appears to have value as a brain injury biomarker, with potential for use in diagnosis, prognosis, and therapeutic monitoring in neurointensive care.

Myelin basic protein (MBP), an abundant protein in white matter, has been examined as a marker of axonal damage in several insults germane to neurointensive care. In TBI, CSF and serum levels have demonstrated excellent specificity, but limited sensitivity [28]. It has also been shown to have value as a serum biomarker in stroke [24••].

Other markers of parenchymal injury such as the astrocyte-specific filament protein glial fibrillary acid protein (GFAP), proteolytic products of spectrin and tau, and synaptophysin have been suggested to have potential utility in neurointensive care [21,36,46,47•]. These markers, however, have been less thoroughly explored than have NSE, S100B, and MBP.

Two recent studies are particularly noteworthy and provide unique insight into the time course and magnitude of changes in three biomarkers, NSE, S100B, and MBP, serially assessed in three key insults in neurointensive care, namely stroke, TBI, and cardiac arrest. First, Jauch *et al.* [24••] assessed the time course of these three markers, along with soluble thrombomodulin in the 359 patients in the US National Institute of Neurological Disorders and Stroke recombinant tissue plasminogen activator (rTPA) stroke study. Higher peak NSE, S100B, and MBP serum concentrations were associated with higher baseline value of US National Institutes of Health Stroke Scale, and peak of both S100B and MBP were associated with lesion size on computed tomography (CT). Similarly, the magnitude of increase in S100B and MBP in the initial 24 h was also inversely associated with favorable outcome. Despite a favorable effect of rTPA, however, there was no difference in early release of biomarkers between treatment and placebo groups. This study represents an important report examining the potential diagnostic use of biomarkers of structural damage in stroke, both related to prognosis, and therapeutic efficacy. A number of potential issues could complicate interpretation of the findings, particularly as they relate to therapy. For example, optimal reperfusion, with a beneficial effect on cellular preservation in penumbral brain regions, might also enhance perfusion of unsalvageable regions and increase transport of brain biomarkers to blood, suggesting a detrimental rather than beneficial effect. Such issues complicate the potential utility of this approach as a therapeutic monitor in stroke, and additional study is needed, both in controlled laboratory models and at the bedside.

Second, Berger *et al.* [48••] assessed NSE, S100B, and MBP in serum from children in three conditions: cardiac arrest requiring rescue breaths or chest compressions, noninflicted TBI from motor vehicle crashes and other accidental causes, and inflicted TBI from child abuse (the shaken baby syndrome). Increases in NSE and S100B in serum were seen in all three groups, but kinetics of the biomarkers varied greatly between groups. S100B peaked early after injury in all three groups and there was no delayed increase, consistent with the lack of delayed astrocyte death. A delayed increase in NSE, however, consistent with delayed neuronal death, was seen in cardiac arrest and inflicted TBI, but not in noninflicted TBI victims. This finding suggests that there is important delayed neuronal death in both cardiac arrest and child abuse victims. It suggests also that there may be an important ischemic component to brain injury in child abuse, in contrast to noninflicted TBI. Increases in MBP were also greatest in children with inflicted TBI, consistent with the possibility of axonal injury from acceleration–deceleration related to violent shaking. One additional caveat from this study was the relative lack of secondary increase in NSE after noninflicted TBI in comparison to cardiac arrest. One interpretation for this finding is the possibility that there is a very limited opportunity for therapies targeting delayed neuronal death in TBI, particularly in centers that already have an aggressive protocol in place for ICP management. Might this be the reason that mild therapeutic hypothermia has been shown to be effective in improving outcome in cardiac arrest but not TBI? Further study is needed since there may be other explanations for this finding. Taken together, these results suggest that serum biomarkers may be very useful to serially monitor patients across insults in neurointensive care, and that expected patterns are seen which are based on known pathophysiological and histopathological findings.

Interrogating the cellular and molecular cascades in secondary injury and repair

We will discuss four approaches to the study of cellular and molecular cascades in the secondary injury and repair response to ischemic or traumatic brain injury, namely, the use of single mediators, proteomics, multiplex technology, and lipidomics.

Use of single mediators for monitoring disease progression or therapeutic efficacy

There are an endless number of studies that have examined single mediators of the secondary injury cascade in human brain injury. One of the most interesting potential mediators that could serve as a marker of damage and therapeutic efficacy is cytochrome c. Studies in humans being treated with chemotherapy for malignancies suggest that cytochrome c release from tumor into serum can be a useful means to rapidly monitor the success of therapy, since apoptosis-mediated cell death in tumors is triggered by cytochrome c release [49]. Cytochrome c release has also been reported as an important mediator of neuronal death in both experimental TBI and cerebral ischemia [50,51]. Recently, Satchell *et al.* [17] reported increases in CSF cytochrome c in pediatric TBI victims. Increases were delayed and generally maximal at several days after injury. In addition, our group is also evaluating this approach in CSF in adults with severe TBI. Thus, this approach could have potential for monitoring the progression of disease in TBI or possibly cerebral ischemia, and one could envisage monitoring other potential cell death or inflammatory mediators depending on the therapy that is being assessed or the specific aspect of the insult that is being targeted.

Proteomic approaches

Diseases such as TBI, stroke, and cardiac arrest set into motion a complex secondary injury cascade and are perfect candidates for molecular monitoring using emerging proteomic approaches, which allow assessment of hundreds or thousands of proteins simultaneously [52–60]. Several studies in experimental models of CNS injury have taken advantage of these approaches and have provided interesting insight into the often cybernetic interactions that occur. Most of this work has been carried out in experimental TBI and stroke. Sironi *et al.* [56] published an initial report using proteomics (two-dimensional gel electrophoresis) to study changes in acute phase proteins in spontaneously hypertensive rats in serum and urine in advance of stroke. A number of inflammatory mediators such as thostatin signaled the development of stroke. A number of other reports followed that applied proteomic approaches in experimental ischemic preconditioning and deep hypothermia circulatory arrest [59,60]. In TBI, the seminal report of Jenkins *et al.* [52] represented the first such approach using two-dimensional gel in TBI and identified over 1500 proteins with 10% demonstrating a 10-fold change between injury and control conditions. This study illustrated the power of this approach by using antibodies that recognized phosphorylation motifs specific to PKB-mediated phosphorylation, allowing simultaneous assessment of a substantial portion of the PKB molecular cascade. The PKB pathway is complex, but is believed to represent an important phosphorylation-dependent cell signaling pathway with predominantly pro-survival effects. Several other reports have followed including studies using small format 2D gel [4], cation/anion exchange chromatography [53], and powerblot [54] to study experimental and clinical TBI. More recently, a two-dimensional gel approach has been used in experimental TBI to begin to explore more delayed changes after experimental TBI and revealed enduring changes in neuronal and glial stress responses, oxidative stress, and

neurotransmitter function, and began to explore the use of this approach to study neuronal plasticity [55].

Use of proteomic approaches in clinical TBI, stroke, and cardiac arrest are more limited. Two reports have recently been published. Gao *et al.* [57] used two-dimensional gel to study CSF from infants with severe TBI and compared pooled samples from patients with inflicted (child abuse) versus noninflicted (accidental) injuries. Making the diagnosis of inflicted TBI in infants is often difficult [35]. Markers of the acute phase response such as haptoglobins did not show the usual marked increase after TBI in the child abuse victims, suggesting the possibility of chronic injury or a delay in presentation in these patients blunting the acute phase response. Haqqani *et al.* [58] studied pediatric TBI patients using isotope-coded affinity tag and tandem mass spectrometry in serum samples. Ninety-five unique proteins were identified, several of which were potentially of brain origin including NSE, amyloid β A4, and α-spectrin. These seminal reports suggest that this approach holds promise, but challenges remain. For example, two-dimensional gel is limited to the study of relatively high copy proteins, and specificity and reproducibility of the other methods, such as powerblot, applied to the complex setting of brain injury remains to be proven.

Multiplex bead technology

Recently, multiplex bead technology has been developed to allow the quantification of multiple proteins in biological samples. This technology, in essence, allows multiple enzyme-linked immunosorbent assay (ELISA) examinations to be performed simultaneously in a single biological sample. In addition, the technique has the advantage of requiring very small amounts of sample. A number of kits are available, generally allowing the assessment of 10–25 related proteins, such as inflammatory mediators, cell death effectors, or growth factors. The multiplex method has been applied to brain tissue in experimental models [61] and recently has been used to assess cytokines and chemokines in CSF from children after severe TBI. The method corroborated individual ELISA on the same samples, and also demonstrated robust increases in a number of cytokines and chemokines after TBI including IL-1β, IL-6, IL-8, IL-10, MIP-1α [62•]. In that report, which assessed samples from children enrolled in a randomized controlled trial of mild therapeutic hypothermia in pediatric TBI, no effect of hypothermia on cytokine levels was reported despite significant effects of cooling on intracranial hypertension. Remarkably, the CSF findings mirrored those of studies in rodent models of TBI, where, despite a beneficial effect of hypothermia, an effect on cytokine markers was not seen [63]. Thus, in addition to assessing the extent of structural degradation and evaluating mechanisms involved in the evolution of damage after injury, the use of proteomic or multiplex technology to study the effect of a treatment on single or multiple mechanistic cascades is promising.

Lipidomic approaches

In addition to assessing protein changes after traumatic or ischemic brain injury, a number of studies have assessed lipid metabolism and the focus has been predominantly on biomarkers of lipid peroxidation, such as F2-isoprostane [64–66]. In addition, new parallel approaches have begun to be applied to study simultaneously, changes in multiple lipids. One such approach has recently been applied to experimental TBI. Bayir *et al.* [67••] in a seminal report in this area used a novel oxidative lipidomics method to study oxidation of key mitochondrial membrane lipids in experimental TBI. Cardiolipin is a phospholipid exclusively found in the mitochondria. A pool of cytochrome c in the mitochondria interacts with cardiolipin and acts as a cardiolipin oxygenase. The oxygenase is activated during apoptosis, uses generated reactive oxygen species and causes oxidation of cardiolipin. The oxidized cardiolipin is required for the release of pro-apoptotic factors, such as cytochrome c, from the mitochondria to the cytosol. As previously discussed, this can trigger the intrinsic apoptosis cascade resulting in neuronal death. After experimental TBI in developing rats, selective oxidation of cardiolipin in brain mitochondria early after injury preceded both cytochrome c release and more delayed nonselective oxidation of other mitochondrial lipids such as phosphatidylserine. This exciting new approach is being explored in clinical TBI in the assessment of CSF and has potential to help unravel the role of lipids in secondary damage after CNS injury.

Conclusion

Given the wealth of reports showing diagnostic, prognostic, and monitoring potential for biomarkers, both those of structural damage in brain and those representing markers of mediators of secondary cascade of injury or repair in the brain, it is long overdue that a panel of markers be implemented into clinical care. Although no single or panel of biomarkers of brain injury has unequivocally passed the specificity test across insults, certainly, markers of hepatic injury such as alanine aminotransferase, aspartate aminotransferase, and alkaline phosphatase, have their own limitations as individual markers, but help guide therapy as a panel in a variety of clinical settings. There are still many unanswered questions in this area of research, however, such as the best statistical methods to analyze the large volume of data generated with many of these methods, the role of intrinsic factors, such as age, sex, and genetic polymorphisms, in contributing to the observed findings, and the relative merits of biomarkers across the spectrum of types and severities of insults in neurointensive care. In addition, issues such as

sample integrity and preservation, normalization, and appropriate control data also must be given careful consideration. Nevertheless, multifaceted cellular, biochemical, and molecular monitoring of proteins and lipids is logical as an adjunct to guiding therapies and improving outcomes in traumatic and ischemic brain injury and we appear to be on the verge of a breakthrough with the use of these markers in neurointensive care.

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References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 217–218).

1 Hossmann KA. Viability thesholds and the penumbra of focal ischemia. Ann Neurol 1994; 36:557–565.

2 Clark RSB, Kochanek PM, Chen M, *et al.* Increases in Bcl-2 and cleavage of caspase-1 and caspase-3 in human brain after head injury. FASEB J 1999; 13:813–821.

3 Zhang X, Graham SH, Kochanek PM, *et al.* Caspase-8 expression and proteolysis in human brain after severe head injury. FASEB J 2003; 17: 1367–1369.

4 Zhang X, Chen Y, Ikonomovic MD, *et al.* Increased phosphorylation of protein kinase B and its substrates after traumatic brain injury in humans and rats. J Cereb Blood Flow Metab 2006; 26:915–926.

5 Rudman D, Fleischer A, Kutner MH. Concentration of 3′, 5′ cyclic adenosine monophosphate in ventricular cerebrospinal fluid of patients with prolonged coma after head trauma or intracranial hemorrhage. N Engl J Med 1976; 295:635–638.

6 McClain CJ, Cohen D, Ott L, *et al.* Ventricular fluid interleukin-1 activity in patients with head injury. J Lab Clin Med 1987; 110:48–54.

7 McClain C, Cohen D, Phillips R, *et al.* Increased plasma and ventricular fluid interleukin-6 levels in patients with head injury. J Lab Clin Med 1991; 118:225–231.

8 Kochanek PM, Clark RSB, Ruppel RA, *et al.* Biochemical, cellular and molecular mechanisms in the evolution of secondary damage after severe traumatic brain injury in infants and children: Lessons learned from the bedside. Pediatr Crit Care Med 2000; 1:4–19.

9 Bell M, Kochanek PM, Doughty LA, *et al.* Interleukin-6 and interleukin-10 in cerebrospinal fluid after traumatic brain injury in children. J Neurotrauma 1997; 14:451–457.

10 Clark RSB, Carcillo JA, Kochanek PM, *et al.* Cerebrospinal fluid adenosine concentration and uncoupling of cerebral blood flow and oxidative metabolism after severe head injury in humans. Neurosurgery 1997; 41:1284–1293.

11 Whalen MJ, Carlos TM, Kochanek PM, *et al.* Interleukin-8 is increased in CSF of children with severe head injury. Crit Care Med 2000; 28:929–934.

12 Clark RSB, Kochanek PM, Adelson PD, *et al.* Increases in Bcl-2 protein in cerebrospinal fluid and evidence for programmed-cell death in infants and children following severe traumatic brain injury. J Pediatrics 2000; 137:197–204.

13 Palmer AM, Marion DW, Botscheller ML, *et al.* Increased transmitter amino acid concentration in human ventricular CSF after brain trauma. Neuroreport 1994; 6:153–156.

14 Amick JE, Yandora KA, Janesko K, *et al.* TH1 versus TH2 cytokine profiles in cerebrospinal fluid after severe traumatic brain injury in children. Pediatr Crit Care Med 2001; 2:260–264.

15 Robertson CL, Minamino N, Ruppel R, *et al.* Increased adrenomedullin in cerebrospinal fluid after traumatic brain injury in infants and children. J Neurotrauma 2001; 18:861–868.

16 Bayır H, Kagan VE, Tyurina YY, *et al.* Assessment of antioxidant reserve and oxidative stress in cerebrospinal fluid after severe traumatic brain injury in infants and children. Pediatr Res 2002; 51:571–578.

17 Satchell MA, Lai Y, Kochanek PM, *et al.* Cytochrome C, a biomarker of apoptosis, is increased in cerebrospinal fluid from infants with inflicted brain injury from child abuse. J Cereb Blood Flow Metab 2005; 25:919–927.

18 Zedler S, Faist E. The impact of endogenous triggers on trauma-associated inflammation. Curr Opin Crit Care 2006; 12:595–601.

19 Vaagenes P, Safar P, Diven W, *et al.* Brain enzyme levels in CSF after cardiac arrest and resuscitation in dogs: markers of damage and predictors of outcome. J Cereb Blood Flow Metab 1988; 8:262–275.

20 Vaagenes P, Mullie A, Fodstad DT, *et al.* The use of cytosolic enzyme increase in cerebrospinal fluid of patients resuscitated after cardiac arrest. Am J Emerg Med 1994; 12:621–624.

21 Pelinka LE, Kroepfl A, Leixnering M, *et al.* GFAP versus S100B in serum after traumatic brain injury: relationship to brain damage and outcome. J Neurotrauma 2004; 21:1553–1561.

22 Savola O, Pyhtinen J, Leino TK, *et al.* Effects of head and extracranial injuries on serum protein S100B levels in trauma patients. J Trauma 2004; 56:1229–1234.

23 Müller K, Townend W, Biasca N, *et al.* S100B serum level predicts computed tomography findings after minor head injury. J Trauma 2007; 62:1452–1456.

24 •• Jauch EC, Lindsell C, Broderick J, *et al.* Association of serial biochemical markers with acute ischemic stroke: the National Institute of Neurological Disorders and Stroke recombinant tissue plasminogen activator Stroke Study. Stroke 2006; 37:2508–2513.

Important work in the field of stroke evaluated time course of three biomarkers, namely S100B, NSE, and MBP along with soluble thrombomodulin in the 359 patients in the NINDS recombinant tissue plasminogen activator (rTPA) Stroke Study. Higher peak NSE, S100B, and MBP serum concentrations were associated with baseline value of NIH Stroke Scale, and peak of both S100B and MBP were associated with lesion size on CT. Similarly, the magnitude of increase in S100B and MBP in the initial 24 h was also inversely associated with favorable outcome.

25 Piazza O, Cotena S, Esposito G, *et al.* S100B is a sensitive but not specific prognostic index in comatose patients after cardiac arrest. Minerva Chir 2005; 60:477–480.

26 Foerch S, Wunderlich MT, Dvorak F, *et al.* Elevated serum S100B levels indicate a higher risk of hemorrhagic transformation after thrombolytic therapy in acute stroke. Stroke 2007; 38:2491–2495.

27 Oertel M, Schumacher U, McArthur DL, *et al.* S-100B and NSE: markers of initial impact of subarachnoid haemorrhage and their relation to vasospasm and outcome. J Clin Neurosci 2006; 13:834–840.

28 Berger RP, Adelson PD, Pierce MC, *et al.* Serum neuron-specific enolase, S100B and myelin basic protein concentrations after inflicted and noninflicted traumatic brain injury in children. Journal of Neurosurgery: Pediatrics 2005; 103:61–68.

29 Berger RP, Janesko KL, Wisniewski SR, *et al.* Neuron-specific enolase and S100B in cerebrospinal fluid after severe traumatic brain injury in infants and children. Pediatrics 2002; 109:e31.

30 Berger RP, Pierce MC, Wisniewski SR, *et al.* Serum S100B concentrations are increased after closed head injury in children: A preliminary study. J Neurotrauma 2002; 19:1405–1409.

31 Jönsson H, Johnsson P, Bäckström M, *et al.* Controversial significance of early S100B levels after cardiac surgery. BMC Neurol 2004; 4:24.

32 Routsi C, Stamataki E, Nanas S, *et al.* Increased levels of serum S100B protein in critically ill patients without brain injury. Shock 2006; 26:20–24.

33 Berger RP, Kochanek PM. Urinary S100B concentrations are increased after brain injury in children: A preliminary study. Pediatr Crit Care Med 2006; 7:557–561.

34 Piazza O, Storti MP, Cotena S, *et al.* S100B is not a reliable prognostic index in paediatric TBI. Pediatr Neurosurg 2007; 43:258–264.

35 Berger RP, Dulani T, Adelson PD, *et al.* Identification of brain injury in well appearing infants using serum and cerebrospinal markers: A possible screening tool. Pediatrics 2006; 117:325–332.

36 Wunderlich MT, Lins H, Skalej M, *et al.* Neuron-specific enolase and tau protein as neurobiochemical markers of neuronal damage are related to early clinical course and long-term outcome in acute ischemic stroke. Clin Neurol Neurosurg 2006; 108:558–563.

37 Selakovic V, Faicevic R, Radenovic L. The increase of neuron-specific enolase in cerebrospinal fluid and plasma as a marker of neuronal damage in patients with acute brain infarction. J Clin Neurosci 2005; 12:542–547.

38 Bandyopadhyay S, Hennes H, Gorelick MH, *et al.* Serum neuron-specific enolase as a predictor of short-term outcome in children with closed traumatic brain injury. Acad Emerg Med 2005; 12:732–738.

39 Beers SR, Berger RP, Adelson PD. Neurocognitive outcome and serum biomarkers in inflicted versus noninflicted traumatic brain injury in young children. J Neurotrauma 2007; 24:97–105.

40 Stålnacke BM, Ohlsson A, Tegner Y, Sojka P. Serum concentrations of two biochemical markers of brain tissue damage S-100B and neuron specific enolase are increased in elite female soccer players after a competitive game. Br J Sports Med 2006; 40:313–316.

41 McKeating EG, Andrews PJ, Mascia L. Relationship of neuron specific enolase and protein S-100 concentrations in systemic and jugular venous serum to injury severity and outcome after traumatic brain injury. Acta Neurochir Suppl 1988; 71:117–119.

42 Grubb NR, Simpson C, Sherwood RA, *et al.* Prediction of cognitive dysfunction after resuscitation from out-of-hospital cardiac arrest using serum neuron-specific enolase and protein S-100. Heart 2007; 93:1268–1273.

43 Auer J, Berent R, Weber T, *et al.* Ability of neuron-specific enolase to predict survival to hospital discharge after successful cardiopulmonary resuscitation. CJEM 2006; 8:13–18.

44 Pelinka LE, Hertz H, Mauritz W, *et al.* Nonspecific increase of systemic neuron-specific enolase after trauma: clinical and experimental findings. Shock 2005; 24:119–123.

45 Pelinka LE, Jafarmadar M, Redl H, Bahrami S. Neuron-specific-enolase is increased in plasma after hemorrhagic shock and after bilateral femur fracture without traumatic brain injury in the rat. Shock 2004; 22:88–91.

46 Shojo H, Kibayashi K. Changes in localization of synaptophysin following fluid percussion injury in the rat brain. Brain Res 2006; 1078:198–211.

47 • Pineda JA, Lewis SB, Valadka AB, *et al.* Clinical significance of alpha-II-spectrin breakdown products in cerebrospinal fluid after severe traumatic brain injury. J Neurotrauma 2007; 24:354–366.
This recent report describes the use of biomarkers of calpain-mediated proteolysis in CSF to assess patients with severe TBI. Simultaneous tracking of markers of calpain proteolysis and caspase proteolysis in biological samples could provide insight into specific cell death pathways, specifically necrosis versus apoptosis, respectively.

48 •• Berger RP, Adelson PD, Richichi R, Kochanek PM. Serum biomarkers after traumatic and hypoxemic brain injuries: Insight into the biochemical response of the pediatric brain to inflicted brain injury. Dev Neurosc 2006; 28 (4–5):327–335.
This enlightening study in the field of pediatric neurointensive care compared the time course of three biomarkers NSE, S100B, and MBP in three important conditions, namely TBI, inflicted childhood neurotrauma (child abuse), and cardiac arrest. Remarkably, evidence in serum of delayed neuronal death was much more prominent in victims of cardiac arrest and inflicted childhood neurotrauma than in noninflicted TBI.

49 Renz A, Berdel WE, Kreuter M, *et al.* Rapid extracellular release of cytochrome c is specific for apoptosis and marks cell death in vivo. Blood 2001; 98:1542–1548.

50 Morita-Fujimura Y, Fujimura M, Kawase M, *et al.* Release of mitochondrial cytochrome c and DNA fragmentation after cold injury-induced brain trauma in mice: possible role in neuronal apoptosis. Neurosci Lett 1999; 267:201–205.

51 Zhao H, Yenari MA, Cheng D, *et al.* Biphasic cytochrome c release after transient global ischemia and its inhibition by hypothermia. J Cereb Blood Flow Metab 2005; 25:1119–1129.

52 Jenkins LW, Peters GW, Dixon CE, *et al.* Conventional and functional proteomics using large format two-dimensional gel electrophoresis 24 h after controlled cortical impact in postnatal day 17 rats. J Neurotrauma 2002; 19:715–740.

53 Kobeissy FH, Ottens AK, Zhang Z, *et al.* Novel differential neuroproteomics analysis of traumatic brain injury in rats. Mol Cell Proteomics 2006; 5:1887–1898.

54 Jenkins LW, Kochanek PM, Dixon CE, *et al.* Acute protein kinase changes after TBI and therapeutic hypothermia in a murine model of CCI using high-throughput immunoblotting. J Neurotrauma 2006; 23:1038–1038.

55 Kochanek AR, Kline AE, Gao WM, *et al.* Gel-based hippocampal proteomic analysis 2 weeks following traumatic brain injury to immature rats using controlled cortical impact. Dev Neurosci 2006; 28:410–419.

56 Sironi L, Tremoli E, Miller I, *et al.* Acute-phase proteins before cerebral ischemia in stroke-prone rats: identification by proteomics. Stroke 2001; 32:753–760.

57 Gao W, Chadha MS, Berger RP, *et al.* Biomarkers and diagnosis: A gel-based proteomic comparison of human cerebrospinal fluid between inflicted and noninflicted pediatric traumatic brain injury. J Neurorauma 2007; 24:45–53.

58 Haqqani AS, Hutchison JS, Ward R, Stanimirovic DB. Biomarkers and diagnosis; protein biomarkers in serum of pediatric patients with severe traumatic brain injury identified by ICAT-LC-MS/MS. J Neurotrauma 2007; 24:54–74.

59 Dhodda VK, Sailor KA, Bowen KK, Vemuganti R. Putative endogenous mediators of preconditioning-induced ischemic tolerance in rat brain identified by genomic and proteomic analysis. J Neurochem 2004; 89:73–89.

60 Sheikh AM, Barrett C, Villamizar N, *et al.* Proteomics of cerebral injury in a neonatal model of cardiopulmonary bypass with deep hypothermic circulatory arrest. J Thorac Cardiovasc Surg 2006; 132:820–828.

61 Hulse RE, Kunkler PE, Fedynyshyn JP, Kraig RP. Optimization of multiplexed bead-based cytokine immunoassays for rat serum and brain tissue. J Neurosci Methods 2004; 136:87–98.

62 • Buttram SDW, Wisniewski SR, Jackson EK, *et al.* Multiplex assessment of cytokine and chemokine levels in cerebrospinal fluid following severe pediatric traumatic brain injury: effects of moderate hypothermia. J Neurotrauma 2007; 24:1707–1718.
This is an initial report of the application of multiplex technology to the study of acute brain injury. In this study, changes in cytokine levels in CSF from infants and children with severe TBI were assessed and the effect of therapeutic hypothermia on the inflammatory response was also evaluated. This work also demonstrates how proteomic approaches can be used to study complex therapies such as hypothermia in clinical practice.

63 Truettner JS, Suzuki T, Dietrich WD. The effect of therapeutic hypothermia on the expression of inflammatory response genes following moderate traumatic brain injury in the rat. Brain Res Mol Brain Res 2005; 138:124–134.

64 Wagner AK, Bayır H, Puccio A, *et al.* Gender differences in the relationship of excitotoxicity and ischemia to oxidative stress after severe TBI. Am J Phys Med Rehabil 2003; 82:232.

65 Bayır H, Marion DW, Puccio AM, *et al.* Marked gender effect on lipid peroxidation after severe traumatic brain injury in adult patients. J Neurotrauma 2004; 21:1–8.

66 Wagner AK, Bayır H, Ren D, *et al.* Relationships between cerebrospinal fluid markers of excitotoxicity, ischemia, and oxidative damage after severe TBI: The impact of gender, age, and hypothermia. J Neurotrauma 2004; 21:125–136.

67 •• Bayır H, Tuyrin VA, Tyurin YY, *et al.* Selective early cardiolipin oxidation after brain trauma: a lipidomics analysis. Ann Neurol 2007; 62:154–169.
This describes initial application of the use of lipidomics in experimental TBI. In this report, oxidative lipidomics was used to show that oxidative damage to mitochondria early after injury was not random but preferentially affects the lipid cardiolipin. This early oxidative damage may result in the release of cytochrome c from the mitochondrial membrane initiating the intrinsic pathway of apoptotic cell death.