

# Serum markers of severe traumatic brain injury: Are they useful?

Linda E. Pelinka<sup>1</sup>, Ludwig Boltzmann<sup>2</sup>

*Scand J Trauma Resusc Emerg Med* 2005; **13**; 113-115

<sup>1</sup> Department of Anesthesiology & Critical Care Medicine, Lorenz Boehler Trauma Center of the AUVA (Austrian Workers' Compensation Board).

<sup>2</sup> Ludwig Boltzmann Institute for Experimental & Clinical Traumatology Vienna, Austria, European Union

## Background

Before attempting to answer whether or not serum markers are "useful", let us first briefly consider the pathophysiology of severe traumatic brain injury (TBI) as we see it in the trauma intensive care unit: one of the main problems is the development of secondary brain damage triggered by both primary trauma and increased intracranial pressure, decreased mean arterial pressure and decreased cerebral perfusion pressure(1). One of the main difficulties the intensivist is faced with is monitoring and managing patients to provide continuous effective critical care and to limit the development of secondary brain damage. Obviously, the more severely injured the patient is, the more difficult monitoring and management may become, i.e. though patients with severe isolated TBI are sometimes difficult to manage, patients with additional multiple trauma can pose an even greater challenge. In recent years, improved intracranial pressure monitoring and modern neuroimaging techniques such as computed tomography and magnetic resonance imaging have contributed greatly to the assessment and care of TBI(2). Nevertheless, for all their accuracy, both techniques are expensive, not always available, and above all are associated with such stress for TBI patients that they cannot be considered suitable for frequent follow-ups. Thus, assessment, monitoring and management of TBI remains difficult for the intensivist and often stressful for the patient. A blood test measuring one or more markers to determine the status quo of brain damage would no doubt be welcome in the setting of severe TBI. Such markers would be useful indeed in guiding critical care and evaluating the prognosis of patients with severe TBI.

Similarly to tests for damage to other organs (i.e. troponin for damage to the heart), a test for damage to the brain might well be based upon the measurement of one (or more) serum marker(s). Ideally, markers of brain damage should be measurable quickly and simply and should be measurable in serum, since serum is more readily available than cerebrospinal fluid(3). Above all, however, ideal markers of brain damage should be both highly brain-specific and sensitive(4).

Since the early Eighties, there has been an increasing amount of research on markers of brain damage. Easily detectable substances derived from neurons and glia were measured by commercially available assays and studied as markers of brain damage in various clinical settings ranging from stroke(5) and cardiac arrest(6) to traumatic brain injury(7). This review will

focus on the three most well-known serum markers, neuron-specific enolase (NSE), S100B and glial fibrillary acidic protein (GFAP).

## Neuron-specific enolase (NSE)

NSE is a glycolytic enzyme with a molecular mass of 78 kD and a biological half-life of 48 hours(8). NSE is found primarily in the cytoplasm of neurones, but also in peripheral neuroendocrine cells and in certain rare tumors associated with amine precursor uptake, such as small-cell lung cancer, neuroblastoma and melanoma. NSE is found in platelets and erythrocytes as well(9). NSE is passively released by cell destruction only – it is not actively secreted into the extracellular space. Serum NSE levels over 10µg/L are considered pathologic(3).

Clinically, we conducted a prospective study on trauma patients admitted to the hospital within the first 8 hours after trauma(10). On the one hand we found that NSE remained slightly to markedly elevated in the non-survivors suffering from TBI, and rose to a peak 24-96 hours before death - showing a relationship to outcome after TBI. On the other hand, we found that NSE was elevated within the first 48 hours after trauma in patients with multiple trauma but without TBI, verified by computed tomography upon admission to the hospital. After the first 48 hours, NSE dropped back to normal values and remained normal in all multiple trauma patients without TBI. Since these multiple trauma patients without TBI all survived, we cannot say whether NSE would have risen to a peak before death as it did in the non-survivors of TBI. In experimental studies we have also found that NSE was increased without any brain damage whatsoever after hemorrhagic shock, open femur fracture and local ischemia and reperfusion of the liver, gut and kidney. NSE has the advantage of being the only marker found primarily in the neurones rather than in glia. However, apart from the above-mentioned problems associated with multiple trauma, NSE has one serious drawback: it is found in erythrocytes and is thus released into the blood during hemolysis(11), i.e. hemolysis may cause a falsely positive NSE increase.

## S100B

S100B is a calcium-binding protein with a molecular mass of 10-12 kD and a biological half-life of about 1 hour(3). S100B

is found primarily in the cytoplasm of astroglia and Schwann cells, but also in non-nervous cells such as adipocytes, chondrocytes and melanoma cells. In contrast to other markers, S100B can be both actively secreted into the extracellular space and passively released by cell death(12). It was termed "S100" because it is partially soluble in a 100% saturated solution of ammonium sulfate(12). Serum S100B levels over 0.2 $\mu$ g/L are considered pathologic(3).

Clinically, S100B has been considered to be a reliable marker of brain damage and a good indicator of outcome for some years(13). Recently, however, evidence has been increasing that S100B is not necessarily a reliable marker of brain damage in every setting: S100B increases have been found without brain injury after both cardiac surgery(14) and trauma(15). In a prospective study of 55 trauma patients (Injury Severity Score, ISS > 23, Glasgow Coma Score, GCS < 8) classified by radiography, computed tomography, ultrasound and neurology as TBI without multiple trauma, TBI with multiple trauma or multiple trauma without TBI, we measured S100B initially after trauma and daily for a maximum of 21 days(16). We found that both survivors and non-survivors had markedly increased S100B levels for 24-48 hours after trauma. S100B levels returned to normal within the first 48 hours after trauma in all survivors. In contrast, we found that all non-survivors of isolated TBI had S100B values which either increased consistently or dropped and then increased again after the initial posttraumatic increase. We did not find any relationship whatsoever between S100B and localization, pattern or severity of TBI. According to receiver operating characteristic curve analysis and calculation of the area under the curve, S100B is equally accurate for mortality prediction at 24, 48 and 72 hours after trauma and most accurate >84 hours after trauma. We found that sensitivity/specificity for mortality prediction were more accurate in TBI without multiple trauma than in TBI with multiple trauma. In other words, though S100B may be a reliable marker of brain damage in TBI without multiple trauma 24 hours after trauma and thereafter, it appears to be less reliable in TBI with multiple trauma.

Experimentally, we have verified that hemorrhagic shock induces a significant S100B increase in serum and that the S100B increase is associated with the severity of shock in rats: S100B is significantly higher in severe shock than in moderate shock (17). Further experimental studies in rats have shown that S100B is significantly increased early after bilateral femur fracture(18) and after local ischemia and reperfusion of the liver, the gut and the kidney(19). These findings clearly indicate that S100B is not a reliable marker of brain damage in the early post-traumatic setting, which is frequently associated with hemorrhagic shock, local ischemia and/or open fractures.

### **Glial fibrillary acidic protein (GFAP)**

GFAP is a filament protein with a molecular mass of approximately 45 kD. GFAP is found primarily in the astroglial cytoskeleton and was therefore formerly known as astroprotein(20). In contrast to S100B and NSE, GFAP is not found outside the central nervous system and is thus considered

to be highly brain-specific(21). Moreover, in contrast to S100B, which is a marker of activation but not necessarily of cell damage, GFAP does indeed appear to be a marker of actual cell damage(22). Serum GFAP levels over 0.033 $\mu$ g/L are considered pathologic(3).

Clinically, we investigated the brain-specificity and relationship of GFAP to brain damage and outcome after TBI. In a prospective study of patients with TBI or with multiple trauma without TBI (verified by computed tomography) we measured serum GFAP at admission and daily during intensive care, and documented computed tomography, daily highest intracranial pressure, lowest cerebral perfusion pressure, lowest mean arterial pressure, and three-month Glasgow Outcome Score (GOS). After TBI, we found that GFAP was significantly higher in non-survivors than in survivors and higher in patients with GOS 1 (death) than GOS 4-5 (moderate to good recovery). Interestingly, we also found that GFAP, in contrast to S100B and NSE, remained completely normal in multiple trauma patients without TBI. GFAP showed a relationship the pattern of TBI in computed tomography and was significantly higher in more severe TBI. From these findings we conclude that GFAP is not only brain-specific, but also related to the severity of brain injury and to outcome after TBI(23).

### **Summary**

Less than 5 years ago, just before the measurement of GFAP was described in serum, S100B and NSE appeared to be extremely promising markers of brain damage and hopes were soaring that quick, simple and inexpensive measurement of S100B and NSE might even prove to be a laboratory technique capable of partially replacing expensive and tedious neuroimaging procedures. Recently, however, both clinical and experimental studies have shown that things are clearly not quite as simple as they once appeared to be.

- Individual NSE and/or S100B levels measured on any given day are of very limited value. The course of NSE and S100B monitored on a day-to-day basis is required to judge the development of secondary brain damage(16).
- S100B (unlike NSE and GFAP) can be a marker of cell activation. S100B is capable of mediating neuronal reorganization and may even have a positive effect upon brain plasticity. Thus, depending upon the time elapsed since TBI, increased S100B levels may in fact be indicating brain cell activation rather than brain cell damage(24).
- The brain specificity of NSE has been challenged, since NSE has been shown to be released into the blood in the absence of TBI during hemolysis(9) and after multiple trauma(10), hemorrhagic shock, open fractures and local organ ischemia and reperfusion.
- The brain specificity of S100B has been challenged, since there is strong clinical and experimental evidence of extra-cerebral sources of S100B(14). S100B is not a reliable marker of brain damage within the first 48 hours after trauma(16), probably due to hemorrhagic shock(17), ischemia(19) and/or long bone fractures(18). Furthermore, S100B is not a reliable marker of brain damage within 8

hours of major surgical procedures such as cardiac surgery, probably due to contamination of S100B from shed blood (6).

- Though preliminary studies indicate that GFAP, the newest and most promising of the serum markers of brain damage, is highly specific for the central nervous system and thus particularly well-suited as a marker for clinical practice(23), further clinical research will be required to support this evidence (3).
- Last not least, one of the main limitations in measuring serum markers is time. S100B and NSE assays require approximately 2 hours until results are ready. Clinical routine relies heavily upon simple, quick assays which provide instant results. Though such instant assays are not available on the market yet, the industry has risen to the challenge and there is hope that instant assays will be commercially available before too long(3). A GFAP assay is not commercially available yet, but routine measurement will be possible in Europe later this year, according to the manufacturer (DiaSorin S.P.A., Saluggia, Italy and DiaSorin Inc., Stillwater, Minn.) In the United States, NSE, S100B and GFAP can be measured in clinical trials, but routine use is awaiting approval by the Food and Drug Administration.

## Conclusion

Serum markers of severe TBI are indeed useful. Though no single brain-specific marker has yet been unanimously established for traumatic brain in routine clinical practice, there is overwhelming evidence that serum markers of TBI are useful in neurointensive care. Presently, S100B is the most widely acknowledged marker, and is definitely useful, provided the intensivist is aware of its strengths and weaknesses(13). It is conceivable that GFAP will become a widely acknowledged marker as well(22) and that future intensivists will be using a combination of markers to assess of primary brain injury, detect ongoing secondary brain injury and possibly even to assess the benefits of neuroprotective drugs (3).

## References

1. Chesnut RM. Avoidance of hypotension: conditio sine qua non of successful severe head-injury management. *J Trauma* 1997; **42**: S4-S9.
2. Kant R, Smith-Seemiller L, Isaac G, Duffy J. Tc-HMPAO SPECT in persistent post-concussion syndrome after mild head injury: comparison with MRI/CT. *Brain Inj* 1997; **11**: 115-124.
3. Ingebrigtsen T, Romner B. Biochemical serum markers of traumatic brain injury. *J Trauma* 2002; **52**: 798-808.
4. Bakay RA, Ward AA, Jr. Enzymatic changes in serum and cerebrospinal fluid in neurological injury. *J Neurosurg* 1983; **58**: 27-37.
5. Elting JW, de Jager AE, Teelken AW, et al. Comparison of serum S-100 protein levels following stroke and traumatic brain injury. *J Neurol Sci* 2000; **181**: 104-110.

6. Jönsson H. S100B and cardiac surgery: possibilities and limitations. *Rest Neurol Neurosci* 2003; **21**: 151-157.
7. Raabe A, Grolms C, Sorge O, Zimmermann M, Seifert V. Serum S100B protein in severe head injury. *Neurosurgery* 1999; **45**: 477-483.
8. Schmechel D, Marangos PJ, Brightman N. Neuron specific enolase is a molecular marker for peripheral and central neuroendocrine cells. *Nature* 1978; **276**: 834-837.
9. Cooper EH. Neuron-specific enolase. *Int J Biol Markers* 1994; **4**: 205-210.
10. Pelinka LE, Mauritz W, Toegel E, Redl H. S100B and NSE as markers of severity and outcome after traumatic brain injury. *Rest Neurol Neurosci* 2000; **16**: 283.
11. Johnson P. Markers of cerebral ischemia after cardiac surgery. *J Cardiothorac Vasc Anesth* 1996; **10**: 120-126.
12. Zimmer DB, Cornwall EH, Landar A, Song W: The S100 protein family: history, function, and expression. *Brain Research Bulletin* 1995; **37**: 417-429.
13. Snyder-Ramos SA, Boettiger B. Molecular markers of brain damage – clinical and ethical implications with particular focus on cardiac arrest. *Rest Neurol Neurosci* 2003; **21**: 123-140.
14. Anderson RE, Hanson LO, Nilsson O, Liska J, Settergren G, Vaage J. Increase in serum S100A1-B and S100BB during cardiac surgery arises from extracerebral sources. *Ann Thorac Surg* 2000; **71**: 1512-1517.
15. Anderson RE, Hansson L-O, Nilsson O, Djilali-Merzoug R, Settergren G: High serum levels for trauma patients without head injuries. *Neurosurgery* 2001; **48**: 1255.
16. Pelinka LE, Toegel E, Mauritz W, Redl H. Serum S100B: a marker of brain damage: in traumatic brain injury with and without multiple trauma. *Shock* 2003; **19**: 195-200.
17. Pelinka LE, Bahrami S, Szalay L, Umar F, Redl H. Hemorrhagic shock induces an S100B increase associated with shock severity in the rat. *Shock* 2003; **19**: 422-426.
18. Pelinka LE, Szalay L, Jafarmadar M, Schmidhammer R, Redl H, Bahrami S. Circulating S100B is increased after bilateral femur fracture without brain injury in the rat. *Br J Anaesth* 2003; **91**: 595-597.
19. Pelinka LE, Harada N, Szalay L, Jafarmadar M, Redl H, Bahrami S. Release of S100B differs during ischemia and reperfusion of the liver, the gut and the kidney in rats. *Shock* 2004; **21**: 72-76.
20. Mori T. Studies on astrocyte specific antigen (astroprotein). *Neurochemistry* (Tokyo) 1970; **9**: 75-78.
21. Missler U, Wiesmann M, Wittmann G, Magerkurth O, Hagenstrom H. Measurement of glial fibrillary acidic protein in human blood: analytical method and preliminary clinical results. *Clin Chem* 1999; **45**: 138-141.
22. Regner A, Alves LB, Chemale I et al. Neurochemical characterization of traumatic brain injury in humans. *J Neurotrauma* 2001; **18**: 783-792.
23. Pelinka LE, Kroepfl A, Schmidhammer R, Krenn M, Buchinger W, Redl H, Raabe A. Glial Fibrillary Acidic Protein in Serum after Traumatic Brain Injury. *J Trauma in press*.
24. Van Eldik LJ, Wainwright MS. The Janus face of glial-derived S100B: beneficial and detrimental functions in the brain. *Rest Neurol Neurosci* 2003; **21**: 97-108.