Serum Biochemical Markers of Brain Injury

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Abstract: The diagnosis and assessment of brain damage is currently based on the clinical examination and the modern neuro-imaging techniques. Electrophysiology, haemodynamic monitoring and invasive neuromonitoring constitute additional tools for monitoring of the brain function and clinical course of the patient. However, despite the substantial progress, clinical and neuro-monitoring methods are quite often not sufficient to evaluate and quantify the severity of the initial and secondary destructive processes and hence they cannot guide efficient therapeutic measures and prognosticate effectively the outcome. During the last decades, researchers and clinicians have focused on specific markers of brain cell damage to improve the diagnosis and monitoring of neurological insults. Lactate dehydrogenase, creatine kinase, neuron specific enolase, have been proposed as potential markers of brain injury. More recently, other glial markers such as the Myelin Basic Protein, the glial fibrillary acidic protein and the S-100B protein have been measured in blood and used as surrogate biochemical markers for brain injury.

This review summarizes published findings on the above brain specific serum biochemical markers with emphasis on those with clinical utility.

Key Words: Brain injury, neurotrauma, stroke, ischaemia, subarachnoid haemorrhage, tbi, brain tumours, brain biochemical markers.

INTRODUCTION

Neurological events like brain trauma, haemorrhage, and ischaemic stroke result in primary damage of neurons and glia that in combination with secondary insults due to hypoxia, hypotension, seizures, sepsis or central fever can have an adverse effect on brain integrity and neurological outcome.

Clinical assessment of brain function [neurological examination, Glasgow Coma Scale for level of consciousness (GCS), pupillary reactivity, Glasgow Outcome Scale for outcome (GOS)], and neuro-imaging techniques [Computed Tomography (CT), Magnetic Resonance Imaging (MRI), Transcranial Doppler (TCD), Positron Emission Tomography (PET)] constitute the common ways for diagnosis and assessment of brain damage. Moreover, electrophysiology [evoked potentials: Brain Stem (BAEP), Somato-Sensitive (SSEP) and Motor (MEP), electroencephalography (EEG)], haemodynamic monitoring [(arterial blood pressure, Jugular vein Oxygen Saturation ($SjvO_2$)], standard invasive neuromonitoring [intracranial pressure (ICP), cerebral perfusion pressure (CPP)] and advanced techniques of invasive neuromonitoring [brain tissue oxygenation (PtO_2) and microdialysis], constitute additional tools for monitoring of the brain function and clinical course of the patient.

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Despite the substantial progress in cerebral neuro-monitoring, clinical and neuro-monitoring methods are quite often not sufficient to evaluate and quantify the severity of the initial injury as well as the ongoing secondary destructive processes and hence they cannot guide efficient therapeutic measures and prognosticate effectively the final outcome. The problem becomes even more prominent with intensive care comatose patients with facial trauma, pre-existing pupillary abnormalities, extra-cranial injuries and incomplete clinical data. Moreover some clinical symptoms such as mydriasis and abnormal motor response develop late and are often signs of major and irreversible brain damage. It is well known that a prompt diagnosis of a neurological deterioration or complication remains a major challenge in clinical practice.

On the other hand, serum biochemical markers offer valuable information regarding the diagnosis of disease of many organs such as troponin for myocardial infarction, PSA for prostate cancer, creatinine for renal failure, CEA for colon cancer, amylase for pancreatitis, CA-125 for ovarian cancer. These markers are relatively specific for the tissues or the function of the organs and provide useful information for the diagnosis, the severity and the course of the disease, the effect of treatment and the outcome of the patient. Other serum biochemical markers such as CRP are not specific for tissues or organs; however they offer information regarding the body reaction in pathological conditions like infection and trauma.

According to Bakay and Ward the ideal serum marker of brain injury should have high specificity for brain, high sen-

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sitivity for brain injury, no age or sex variability, and rapid appearance in serum, as well as should ensure a predictable relationship between the serum concentration and the tissue injury, show clinical relevance and reliable assays for measurement should be available [1].

Since the nineteen seventies many serum biochemical markers have been proposed as potential brain injury indicators:

SERUM BIOCHEMICAL MARKERS FOR BRAIN INJURY: CHARACTERISTICS AND RELIABILITY

Creatine Kinase (CK) is one of the early serum enzyme markers measured in patients with brain injury. It is an enzyme catalyzing the high potential transfer of a phosphotyl group from creatine phosphate (the tissue reservoir of high-potential phosphoryl groups) to ADP to form ATP (fuel for muscles, heart and brain). It is a 40-53 kDa dimer of two kinds of subunits: M & B. Consequently CK has three isoenzymes: The CK-MM (muscle type), the CK-MB (heart type) and the CK-BB (brain type) [2, 3]. The brain type is located in the astrocytes, however is also presented in organs such as the large intestine, the stomach, the urinary bladder and the prostate gland. According to Table **1** the concentration of the enzyme in these organs is one third to one fourth of that in brain [4, 5].

CK-BB upper normal limit in blood is 3.0 μg/L. It is released after brain tissue injury and the serum levels are higher during the first hours and then fall to normal levels.

CK-MB has been used as serum marker for the isheamic heart disease. Its blood levels increases 4-8 hs following an isheamic attack and normalize 3-4 days later on.

A number of studies reported increased serum CK-BB levels after head injury [6, 7]. Bakay and Ward studying patients with head injury observed a weak correlation between CK-BB and injury severity and they mention that this marker has inadequate sensitivity and specificity [1]. Further studies from Skogseid *et al.* and Levitt *et al.*, revealed the deficiency of CK-BB as a predictor of intracranial injury revealed by CT scan [8, 9].

Lactate Dehydrogenase (LDH) is another early serum biochemical marker for brain injury. It is a cytoplasmic enzyme participating in the glycolysis pathway and transforming pyruvate to lactate, when amount of oxygen is limiting. It is a 140 kDa tetramer of two kinds of 35 kDa subunits: the H type (predominates in the brain and the heart) and the ho-

Table 1. Relative Concentrations of CK-BB, NSE and S-100B Protein in Human Tissues (Markers' Brain Concentration: 100; all other Values in Relation to Brain Concentration). Both NSE and S-100B Present with High Brain Tissue Specificity. Especially, in S-100B Case the Highest Brain Injury Specificity is Combined with Increased Clinical Utility [51-84]

	CK-BB	NSE	S-100B
Brain	100	100	100
Large intestine	49,1	1,9	2,5
Stomach	35,3	2,6	0,7
Urinary bladder	35,3	2,6	0,7
Prostate gland	31,9	2	0,1
Small intestine	19,2	1,9	2,1
Uterus	22,1	1,1	0,2
Vessels	12,1	1,4	0,2
Thyroid gland	11,3	2,6	0,2
Gallbladder	5,4	0,9	1,7
Kidney	5,7	0,1	0,3
Lung	3,5	1,5	0,2
Mammary gland	0,5	0,1	1,8
Spleen	0,7	2,5	1,8
Aorta	0,8	0,5	0,1
Liver	0,3	0,2	0,1
Skeletal muscles	0,3	0,2	0,7
Heart	0,1	0,1	0,2

CK-BB: Creatine Kinase isoenzyme BB, NSE: Neuron Specific Enolase.

mologus M type (predominates in skeletal muscle and the liver). These subunits associate to form five types of tetramers: H_4, H_3M, H_2M_2, H_1M_3, and M_4 making five different isoenzymes (type 1-5). LDH 1 - 3 are found in high concentration in the brain and the heart and LDH 4 and 5 are predominant in skeletal muscles and liver. The type 1 isoenzyme (H_4) has higher affinity for substrates and is allosterically inhibited by high levels of pyruvate. Consequently, type 1 isoenzyme (H_4) is designated to oxidize lactate to pyruvate, which is then utilized as a fuel by the brain and the heart. In contrast, type 5 isoenzyme (M_4) is optimized to operate the reverse direction, to convert pyruvate to lactate to allow glycolysis to proceed under anaerobic conditions particularly in muscles. The others isoenzymes have intermediate properties depending on the ratio of the two kinds of chains [2,3].

LDH 1 has been extensively used as serum marker for the heart isheamic disease. Its blood levels increases 24-48 hs following an isheamic heart attack with maximum levels during the 3rd and 4th day. The normal serum value for LDH type 1 is less than 280 U/ml. LDH 2 & 3 blood levels increase in pulmonary embolus.

Bakay and Ward investigated the role of LDH 1 as serum brain marker in patients with severe and moderate head injury found no correlation between LDH 1 levels and outcome. They concluded that LDH has limited sensitivity and specificity for brain injury [1].

Glial Fibrillary Acidic Protein (GFAP) is the principal intermediate filament in glial cells of the CNS and represents a significant part of the cytoskeleton of the mature astrocytes [10]. It is a monomeric molecule which has been recently measured in blood and used as surrogate biochemical marker for brain injury. Its normal serum concentration is less than 0.04 μg/L and its molecular mass is 40-53 kDa [11].

Missler *et al.*, found increased serum GFAP levels after severe head injury. In addition Pelinka *et al.*, confirmed that GFAP is released after traumatic brain injury (TBI), is related to brain injury severity and outcome after TBI, and is not released after multiple trauma without brain injury [11, 12]. Nylén *et al.*, agreed that serum GFAP is increased during the first days after a severe TBI and is related to clinical outcome [13]. Moreover, Herrmann *et al.*, showed that GFAP could be used as a tool of clinical stroke management and Nylen *et al.*, showed that GFAP provides information about brain injury severity and outcome after subarachnoid haemorrhage [14, 15]. All the above studies confirm that serum GFAP could be a promising biochemical marker for brain injury detection and prognosis.

Myelin Basic Protein (MBP) is found in oligodentroglial cells and appears to be bound to the cellular membrane of central myelin mainly. It is well known that oligodendrocytes are responsible for the formation of the myelin sheaths in the Central Nervous System. Their plasma membrane becomes wrapped around the neural axon. Its molecular mass is 18.5 kDa [2].

MBP can be released into serum after brain injury or demyelinating disease. In control cases the myelin basic protein serum levels was found to range from undetectable to a maximum of 17 μg/L, with mean value of 7.2 μg/L. Thomas *et al.*, found higher serum MBP levels in patients after severe head injury with poor outcome than in those with favorable outcome [16, 17]. Similar findings were reported by Yamazaki *et al.* [18]. It is obvious that further studies are required to clarify the reliability of serum MBP as a serum marker for brain injury.

Neuron Specific Enolase (NSE) is a member of a glycolytic enzymes family (enolases). They are presented as dimeric isoenzymes made of three subunits α, β and γ chains. The γγ and αγ isoforms are referred as NSE and is found in neurons, peripheral neuroendocrine tissue and tumours with amine precursor uptake and degradation function. It is located in the cytoplasm of neurons and is involved in chloride levels balance during the onset of neural activity [19]. Table **1** confirms the high brain specificity of the NSE.

The molecular mass of NSE is 78 kDa with normal serum concentration less than 12.5 μg/L and biological halftime more than 20 hours. NSE has been used as a marker for tumours, such as small cell lung cancer, neuroblastoma & myeloma (amino precursor uptake & degradation system).

Many studies observed increased serum levels of NSE after head injury, although others failed to detect differences between patients and controls [20, 21].

Both McKeating *et al.* and Woertgen *et al.*, reported secondary serum NSE increases particularly in patients with brain injury and unfavorable outcome [22, 23]. However, the aforementioned studies found conflicted results as far as the correlation between the NSE levels and the GCS scores concerns, the correlation between the NSE levels the admission CT scan findings concerns and the correlation between serum NSE levels and the clinical outcome concerns. In addition no relation found between NSE and ICP [23]. Furthermore, Missler *et al.* showed that NSE failed to predict accurately the infarct volume and the long-term neurological outcome in patients with acute ischemic stroke [24]. Consequently, NSE use in clinical practice seems to be quite doubtful.

S-100B protein constitutes a big family of at least 20 proteins of low molecular weight (9-13 kDa) with calcium binding ability that have been identified sharing various degrees of amino acid homology (25-65%) and being mainly characterized by two different Ca^{2+} binding domains (helix-loop-helix) uniformly described as EF-hands [25].

In 1965, Moore identified and named S-100 due to its solubility in a 100% saturated ammonium sulphate solution. It was purified from bovine brain and defined as brain specific [26]. Later S-100 was shown to constitute a homo or hetero dimer of two distinct but related proteins: S-100A1 and S-100B with a molecular weight of approximately 21 kDa and with four Ca^{2+} binding sites [27, 28]. It also binds copper at 4 binding sites and Zn2+ at 6-8 binding sites and such binding influences the Ca^{2+} - binding capacity of the protein [29].

In 1995, a new nomenclature was introduced for S-100 proteins, after the identification of the chromosomal localiza-

tion of 9 members of the S-100 family (S-100A1 – S-100A9) on the long arm of the human chromosome 1 (1q21). According to this, the protein previously called S-100 alpha (S-100a) is now named S-100 A1 (S-100A1). The same nomenclature was also applied for S-100 proteins located on different chromosomes: S-100B (formerly S-100beta), on chromosome 21 (located at a distance of 100-140 kb from the chromosome terminus) and S-100P on chromosome 4 [30-32]. Further members of the S-100 family (S-100A10 – S-100A14) were recognized later [33-35]. Within cells, S-100 it is found as homo-dimers (S-100B / S-100B) or hetero-dimers (S-100A1 / S-100B) of two different subunits (A1 and B) [29].

Types S-100AB and S-100BB are described as S-100B protein and are shown to be highly specific for nervous tissue (Table **1**). It is most abundant in the cytosol of glial cells of the central and peripheral nervous system (astrocytes and Schwann cells) and is also expressed in melanocytes, adipocytes and chondrocytes, although in very low concentrations [36-38 & Table **1**].

Its short biologic half-life makes measurements crucial in the emergency and intensive care settings [39, 40]. Wiesmann *et al.* showed that serum S-100 has no age or sex variability [41]. S-100B serum concentrations are not influenced by blood alcohol or acute alcohol intoxication [42-44]. It can be measured in arterial and venous serum, is not affected by hemolysis and remains stable for several hours, without the need for immediate analysis. It is metabolized and excreted from the kidneys [45, 46].

S-100B could be measured with various techniques [47]. Values above 0.5 μg/L are considered "pathological" and 0.15 – 0.5 μg/L "borderline" according previous studies and the recommendations of the commercial representative [48]. The multiple intracellular and extracellular regulatory activities of the multi-genic S-100 protein family have been extensively described in the literature [49, 50].

High serum S-100B values were found to be strongly correlated with the severity of the primary severe brain trauma, the Glasgow Coma Scale score, the patients' outcome, the neuroradiological findings (CT) and the ICP increase according to many studies [22, 51-63]. Interestingly, Dimopoulou *et al.* mentioned the high predictive value of initial S-100B serum levels in trauma – induced brain death [64]. Serum S-100B protein was also found to be a sensitive biochemical marker of the brain tissue after mild head trauma. Initial increased value is followed by a rapid decline, as expected by the S-100B half-life [65-69].

Moreover, S-100B protein has been found to be elevated after ischaemic stroke. The amount of S-100B released over time is significantly correlated to the infraction volume [14, 24, 70-73]. In addition, serum S-100B protein might be a good prognostic marker for cerebral injury in term newborn infants with hypoxic ischaemic encephalopathy and early detection of intraventricular haemorrhage. It may also represent an index of cerebral cell damage in the perinatal period [74-79]. Moreover, serum S-100B was found to be an indicator of the haemorrhage severity (WFNS scale, CT) as well as a prognosticator of outcome in patients with subarachnoid haemorrhage [80-82].

Finally, serum S-100B measurements were found to be predictor for postoperative deterioration in patients undergoing meningioma resection and a useful prognostic variable in patients with cerebral gliomas [83, 84].

Despite all these, the question of extracranial effect on S-100B release has been raised. However, extra-cranial trauma seems to have a minimal contribution to the measured serum S-100B after severe traumatic head injury [85-88]. A more critical analysis of its role has been attempted by Kleindienst and Ross Bullock [89].

Brain and Heart type Fatty Acid-Binding Proteins, (B-FABP and **H-FABP)** are small 15kDa cytoplasmic, nonenzymatic proteins involved in the intracellular buffering and transport of long-chain fatty acids. B-FABP was first identified in the brains of rodents and showed diverse tissue production during development. In adult-stage mice, B-FABP is produced in very low concentrations and is detected only in glial cells of the white matter. In contrast, H-FABP is detected in the neurons of the gray matter in mice and rats and constitutes 0.01% of total brain cytosolic protein [90].

FABPs are released rapidly from damaged brain cells into the circulation and are cleared from the circulation by the kidney with a plasma half-life of 20 min. In a study with 130 patients with mild traumatic brain injury both markers were found to be elevated in more cases than when S-100B and NSE were used, suggesting higher sensitivity for detection of brain injury [90].

Tau proteins are microtubule-associated proteins that are abundant in neurons in the central nervous system compared to non-neuronal cells. They have a molecular weight of approximately 62 kDa and interact with tubulin to stabilize microtubules and promote tubulin assembly into microtu-bules. They are active primarily in the distal portions of axons where it provides microtubule stabilization but also flexibility as needed. Bulut *et al.* investigating serum tau levels in patients with mild TBI found that they were increased. They concluded that this biomarker may prove helpful in identifying high-risk patients with mild TBI [91].

In an attempt to compare the clinical significance of the aforementioned brain markers Vos *et al.* showed that serum S-100B level >1.13 microg/L was the strongest predictor of death, in comparison with NSE and GFAP [92]. Interestingly, Pelinka *et al.* showed that GFAP was found to be more accurate for early mortality prediction (<12 hs) after TBI and S-100B was more accurate during the later stage when the focus is on monitoring for secondary neurological complications [93]. Berger at al reported that serum marker such as NSE, S100B and MBP are increased in the majority of children with acute TBI [94]. Nylén *et al.* hypothesised that the S-100BB dimer should be better related to outcome after severe traumatic brain injury than S-100A1B or the "sum S-100B concentration". They found that both S-100A1B and S-100BB were related to outcome after severe traumatic brain injury and it seems unlikely that separate

analyses of the dimers are of any advantage compared with measuring S-100B alone [95].

SURROGATE INFLAMMATORY SERUM BIOCHEMICAL MARKERS

The **70 kilodalton heat shock proteins** (**Hsp70**s) are an important part of the cell's machinery for transmembrane transport of proteins and help to protect cells from stress. In particular they are strongly upregulated by thermal or oxidative stress. da Rocha *et al.* in their recent study concluded that increased serum Hsp70 levels may constitute an early predictor of unfavorable outcome in patients with severe TBI [96].

RANTES is an acronym for **Regulated on Activation, Normal T Expressed and Secreted**. It is also known as CCL5. It is an 8kDa protein classified as a chemotactic cytokine or chemokine. It is chemotactic for T cells, eosinophils, and basophils, and plays an active role in recruiting leukocytes into inflammatory sites. With the help of other particular cytokines that are released by T cells, RANTES also induces the proliferation and activation of certain natural-killer (NK) cells. Lumpkins *et al.*, found that RANTES was a significant early marker of severe TBI in critically injured trauma patients, consistent with animal models [97].

Tumor necrosis factor alpha (TNF-alpha), intercellular adhesion molecule-1 and **matrix metalloproteinase** were found to contribute to the outcome of brain ischemic stroke. Sotgiu *et al.* showed a direct significant correlation of the above serum markers with brain infarct size and National Institutes of Health (NIH) scales at stroke onset and 3-month follow-up [98].

We need to clarify that most of the aforementioned markers have been isolated and measured in other biological fluids such as cerebrospinal fluid, amniotic fluid, urine and human milk [99-100]. In addition, a variety of markers such as alpha-II-spectrin breakdown products, glutamate, taurine, free fatty acids, cytochrome c, heat shock protein 60, nitrotyrosine etc have been isolated in the cerebrospinal fluid and found to be useful predictive markers of outcome in patients with brain injury. These markers with increased sensitivity may be applicable to serum testing in the near future [101-107]. It's obvious that serum offers several advantages over cerebrospinal fluid, including ease of accessibility and reduced risk to the patient.

CONCLUSIONS

A variety of serum biochemical markers for brain dysfunction have been investigated and used, although interest in some of these such as CK-BB and LDH 1 has been short lived. On the other hand there are many promising results from clinical studies in particular as far as the GFAP, the MBP and the S-100B protein concern. It is obvious that the literature regarding the S-100B protein is quite more extensive. On the other hand minimal literature is available for FABP, tau proteins, Hsp70s, RANTES, and other serum inflammatory factors and their role as serum markers of brain damage. The assessment of the primary injury and the detection of ongoing secondary damage during intensive care seem to be the most promising clinical applications. However, further clinical and experimental studies have to be performed in order to identify new more reliable serum brain biochemical markers, clarify their precise release mechanisms from damaged cells (glial cells or neurons) through the brain blood barrier and validate their clinical utility.

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