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Review

Biomarkers of Brain Injury in Cerebral Infections

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BACKGROUND: Central nervous system (CNS) infections present a major burden of disease worldwide and are associated with high rates of mortality and morbidity. Swift diagnosis and initiation of appropriate treatment are vital to minimize the risk of poor outcome; however, tools are lacking to accurately diagnose infection, assess injury severity, and predict outcome. Biomarkers of structural neurological injury could provide valuable information in addressing some of these challenges.

CONTENT: In this review, we summarize experimental and clinical research on biomarkers of neurological injury in a range of CNS infectious diseases. Data suggest that in both adults and children, the biomarkers S100B and neuron-specific enlose (NSE), among others, can provide insight into the pathophysiology of CNS infection and injury severity, evolution, and response to treatment. Research into the added utility of combining a panel of biomarkers and in assessing biomarker association with clinical and radiological outcomes warrants further work. Various factors, including age, the establishment of normative values, and comparison of biomarker concentrations across different testing platforms still present challenges in biomarker application.

SUMMARY: Research regarding the value of biomarkers in CNS infections is still in its infancy. However, early evidence supports their utility in diagnosis and prognosis, and potentially as effective surrogate end points in the assessment of novel interventions.

Central nervous system (CNS)[2] infections are an important public health concern worldwide, because they occur commonly and are associated with high rates of mortality and morbidity *(1, 2)*. Management of these conditions remains challenging because clinicians lack the tools to make swift, definitive diagnoses, to assess injury severity, and to predict outcomes *(3–5)*. Most deaths related to CNS infections are the result of cerebral tissue injury. Neurological sequelae in survivors include physical, sensory, and cognitive disability, all of which may be permanent *(1, 6–8)*. Neural injury stems from direct bacterial toxicity and the host inflammatory response *(6, 7)*. Once bacteria have entered the CNS the process of replication and autolysis results in components of the bacterial wall being released into the subarachnoid space, triggering an inflammatory response characterized by the infiltration of immune cells and exudate formation *(6, 7)*. The exudate contributes to the obstruction of normal cerebrospinal fluid (CSF) flow, which may lead to hydrocephalus and raised intracranial pressure, compromised cerebral blood flow (CBF), and ischemic injury. It further coats the cerebral arteries, leading to vasospasm, vasculitis, and occlusion, with resulting ischemia and potential cerebral infarction *(7)*. However, several uncertainties about the disease process remain because the pathogenetic mechanisms of tissue injury are still not fully understood. Quantification of the degree of cerebral injury is inexact: disease severity is commonly evaluated by assessing the clinical status of the patient and radiological manifestations. Yet several factors, both reversible and irreversible, may contribute to the presenting neurological status, and imaging findings manifest late in the disease course, usually once the damage is already permanent. Biomarkers to diagnose disease, quantify injury, and monitor progress are used frequently for other organ systems, but their use in CNS pathology is in its infancy. Several studies have examined biomarkers of neurological tissue injury in different CNS pathologies which shed light on the underlying pathophysiological processes while they are occurring; however, data are sparse. We have reviewed the available literature on biomarkers in cerebral infection.

Role of Biomarkers in CNS Pathology

Biomarkers are measurable objective indicators of normal function or pathology. They provide information about dynamic processes and pathogen activity that assist diagnosis, prognostication, and evaluation of treatment safety and efficacy. They can also act as sur-

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[2] Nonstandard abbreviations: CNS, central nervous system; NSE, neuron-specific enlose; CSF, cerebrospinal fluid; CBF, cerebral blood flow; BBB, blood–brain barrier; TBI, traumatic brain injury; TBM, tuberculous meningitis; MBP, myelin basic protein; GFAP, glial fibrillary acidic protein; NFL, neurofilament protein.

rogate markers for clinical or research end points, such as the effectiveness of novel treatments *(9)*. Their quantification is user independent, and several biological fluids like blood and urine are easily obtained for investigation *(10–13)*.

Biomarkers for CNS pathologies are gaining increasing attention and are being investigated across a spectrum of acute and chronic CNS diseases *(14)*. CNS infection, trauma, hypoxia, inflammation, or degeneration results in cell damage and a collection of breakdown products in the cerebral extracellular fluid as well as increased permeability of the blood–brain barrier (BBB). Diffusing along concentration gradients into the CSF and through a leaky BBB into the bloodstream, these products become accessible measurable indicators of brain injury. The degree to which these biomarker concentrations are increased reflects the severity of injury; the cell specificity hints at the nature and, potentially, the location of injury; and sequential sampling provides information about the evolution of the damage *(9, 15, 16)*.

Ideal biomarkers for brain injury should demonstrate high sensitivity and specificity for the brain. Their release should be associated with irreversible brain injury and reflect the temporal profile of that injury. They should appear rapidly in serum, demonstrate limited variability based on age and sex, and should be easily and speedily quantified by reliable assays *(17)*. However, the task of finding such ideal biomarkers for the brain presents many challenges. The brain is a highly complex and heterogeneous organ with multiple cell types, and disease of the brain varies both in form and severity. CSF is better than serum in reflecting changes in the brain, but is not always accessible. The size and amount of the biomarker infiltrating the blood stream is limited by the BBB, and so serum values may be a function of cell injury as well as the degree of BBB disruption, which commonly occurs in brain injury *(10)*. Even if CSF variability exists, biomarker concentrations may be influenced by the distance between the affected area and the CSF compartment, regional variability of biomarker proteins in the brain, and degradation by proteinases in the parenchyma or CSF *(18)*. In addition, biomarker analysis is purely a quantitative measure which cannot reflect both the qualitative and quantitative functions of the brain *(19)*. One way of overcoming some of these limitations is by using a panel rather than individual biomarkers and by combining these with clinical and radiological tools *(10, 20)*.

Studies in traumatic brain injury (TBI) *(19, 21)*, subarachnoid hemorrhage *(16, 22)*, dementia *(23)*, Alzheimer disease *(24)*, stroke *(16)*, cardiac arrest *(25)*, and various other pathologies have found that biomarkers of CNS injury hold promise as diagnostic and prognostic markers. Their role in infections of the CNS has generated less research. From the available studies, however, 2 key biomarkers with potential have emerged, namely S100B and NSE. In this review we focus primarily on these proteins but also briefly summarize studies on other potential biomarkers.

LIMITATIONS OF BIOMARKERS

Biomarker analysis is prone to some methodological pitfalls. The technique with which samples are collected has implications for their suitability; for example, hemolyzed blood samples may be contaminated by biomarkers released from erythrocytes *(26)*. The collection tube may influence biomarker concentrations, and tubes appropriate to the biomarker of interest should be used *(27)*. Samples should preferably be stored at −70 °C as early as possible to prevent antigen degradation; however, this varies as a function of biomarker stability. Multiple freeze–thaw cycles and prolonged storage can also lead to biomarker breakdown *(28)*. Currently the primary method to test for biomarkers is the immunoassay, which demonstrates good sensitivity and is simple and inexpensive to administer, and for which various kits are commercially available. This technology exploits the high affinity and specificity with which antibodies bind to their antigens, ensuring that only the target antigen in a sample will bind, even when it is present in very low concentrations and in the presence of many other analytes. Immunoassay plates are coated with antibodies for the biomarker of interest; when test samples are added to the plate, the target antigen (biomarker) binds to the antibody and the degree of binding provides a measure of the biomarker present. Because the interaction of antibody and antigen is not associated with a quantifiable physical or chemical change, the binding event is measured by an auxiliary reaction in which one of the immunoreactants is labeled with a substance that can easily be detected by spectrophotometry *(29)*. Platforms differ on the basis of the antibody and label used to detect the antibody–antigen binding; commonly used options include enzymes (ELISAs and RIAs), fluorophores (immunofluorometric assays), and chemiluminescent compounds (chemiluminescence immunoassay). Electrochemical immunoassays, which are label free and rely on changes in charge densities or conductivities to detect antibody–antigen binding, are also becoming increasingly available *(29–31)*. Upper and lower detection limits are variable for different assays, and therefore comparison of reference values and pathology-related measurements across testing platforms is challenging, highlighting the need for the establishment of standardized operating and testing procedures. Large-scale multicenter trials would enable accumulation of sufficient samples for well-powered studies to establish stan-

dards, reference values, and appropriate disease-specific cutoff values. Much biomarker work is still confined to dedicated laboratories or projects; however, the true translation of biomarkers from bench to bedside requires the development of rapid, user-friendly, technologically undemanding tests that can be used on demand at the hospital and clinic levels *(10)*.

S100B

S100B belongs to the larger S100 family of small acidic proteins approximately 10–12 kDa in size and composed of subunits A and B *(32)*. These proteins regulate intracellular processes, including cell growth, transcription, and differentiation. The extracellular concentration of S100 proteins determines their activity; at nanomolar concentrations they have trophic effects, whereas at micromolar concentrations they may cause cell damage and apoptosis. In the CNS, subunit B is found in the highest concentrations *(32)* and is synthesized by astrocytes, oligodendrocytes, and Schwann cells *(33)*. It is involved in cell-to-cell communication *(34)*, cell growth, and intracellular signal transduction, as well as the development and maintenance of the CNS. Several S100B assays are available which detect both S100AB and BB, but currently a reference interval for S100B remains uncertain. Laboratories performing S100B assays are therefore required to generate reference intervals for their population, and studies in patients should include a representative control group. Brain injury results in leakage of S100B into the CSF and passage into the bloodstream through transient disruption of the BBB or via CSF circulation *(15)*. S100B is metabolized by the kidneys and excreted in the urine. Concentrations usually normalize within 24 h after an acute injury; therefore, persistent increases in S100B may reflect ongoing or secondary cellular injury *(35, 36)*. At increased concentrations, S100B may have a neurotoxic effect by inducing apoptosis, causing the release of proinflammatory cytokines as well as nitric oxide from astroglial cells, and contributing to oxidative stress *(1, 33, 34, 37)*. Therefore, increased concentrations not only reflect tissue damage but may also exacerbate it *(37)*. This protein is easily quantified in various biological samples, including CSF, blood, and urine. It remains stable over a wide range of temperatures (room temperature to −70 °C) for up to 48 h after collection and does not degrade after freezing, making it a suitable candidate for daily laboratory measurement and batch analysis in studies *(38, 39)*.

S100B is increased in various CNS pathologies including TBI *(19, 21)*, Alzheimer disease *(24)*, dementia *(23, 32)*, stroke *(40, 41)*, and subarachnoid hemorrhage *(22)*. Extracranial sources of S100B include white and brown fat, skin, skeletal muscle, melanocytes, and adipocytes *(12, 42–44)*. Clinically, increased blood concentrations are reported in melanomas or long bone injuries *(12, 15)*.

REFERENCE VALUES

S100B concentrations differ across various developmental phases from the fetal period through to old age *(45)*. Stages of fetal development are associated with differential S100B concentrations in amniotic fluid and cord blood, suggesting that increased S100B within a physiological range may represent its neurotrophic role in brain maturation *(46, 47)*. Age-related variations in S100B concentrations are, therefore, probably due to the role of S100B in the maturation of glial cells, formation of synapses, and general brain morphogenesis, which occur most frequently during early childhood *(48)*. The pattern of age-related differences in the existing literature is difficult to interpret, however. Portela et al. *(48)* collected serum samples from 3 age groups of healthy donors: neonates, children (4–16 years), and adults (18–70 years). They found no differences based on sex, but they demonstrated that age had a significant negative correlation with S100B, with the highest values found in neonates. After the age of 20 years, the baseline concentrations seemed to stabilize. Gazzolo et al. *(49)* also found that S100B concentrations in serum were associated with age in a detailed study of 1004 children between the ages of 0 and 15 years. Highest values were recorded during the first year of life and then again between 7 and 13 years. The authors hypothesize that these epochs correspond to the greatest spurts of growth and maturation; infancy and early adolescence. Different peaks of S100B based on sex were also found, again a possible manifestation of the growth phenomenon, which varies between the sexes. Bouvier et al. *(50)* found that serum S100B concentrations decreased with age. Their results in an exclusively pediatric cohort showed that the highest concentrations of S100B were recorded before the age of 2 years. The permeability of the BBB, higher protein turnover in neuronal cells, and low renal secretion of S100B, as well as dynamic CNS development in children under 2 years old are possible explanations proposed for this finding. However, Spinella et al. *(37)* found a positive correlation between age and CSF S100B in their study on 107 pediatric patients ranging in age from 3 days to 17.8 years. Although a significant positive correlation was found between age and S100B, the amount of overall variance attributable to age was only 4%. These results have been mirrored in a study of adults conducted by Nygaard et al. *(51)*, who found that S100B in the CSF increased with age, with 43% of the variability attributed to age in men, and 30% in women. Overall S100B concentrations were also significantly higher in men. S100B could not be detected in

Table 1. S100B reference intervals (μg/L).

Cohort and reference	Age group	n	Specimen	Mean (SD)	Median	Range[a]
Pediatric cohort						
Bouvier et al. *(50)*[b]	0–2 years	139	Serum	0.21 (0.12)		0.07–0.83
	>2 years	97		0.11 (0.03)		0.07–0.2
Gazzolo et al. *(49)*[b,c]	0–1 year	85	Serum		0.95	0.44–2.55
	2–7 years	461			0.73	0.44–1.06
	9–11 years				1.65	0.91–1.74
	11–12 years	32			0.45	0.39–0.45
	13–14 years	35			1.23	1.12–2.01
	14–15 years	18			0.78	0.5–0.87
Spinella et al. *(37)*	<1 month	16	CSF		0.79	0.52–1.32
	1–2 months	29			0.6	0.44–0.84
	2–3 months	11			0.61	0.4–0.81
	3–12 months	20			0.58	0.46–0.73
	1–5 years	10			0.96	0.77–1.33
	5–10 years	8			0.87	0.65–1.07
	10–15 years	7			1.3	0.87–1.8
	15–18 years	6			1.2	0.66–1.8
Mixed cohort						
Portela et al. *(48)*	Neonates	19	Serum		1.79	1.57–2.44
	4–9 years	16			0.37	0.27–0.41
	10–15 years	8			0.31	0.26–0.37
	16–20 years	10			0.11	0.02–0.2
	21–25 years	13			0.1	0.05–0.15
	26–30 years	9			0.1	0.06–0.15
	30–70 years	54			0.06	0.03–0.1
Van Engelen et al. *(4)*[d]	1 year		CSF			0.9–2.6[e]
	20 years					1.1–3.3
	40 years					1.3–4
	60 years					1.6–5
Adult cohort						
Wiesmann et al. *(52)*	18–65 years	200	Plasma		0.05	0.02–0.1[f]
Nygaard et al. *(51)*	20–89 years	110	Serum	Undetectable		
			CSF	1.9 (0.7) (males) 1.5 (0.5) (females)		

[a] Range is the 25th to 75th percentile unless otherwise stated.
[b] Additional age-related data available in original articles.
[c] Sample sizes derived from article where possible.
[d] Sample sizes not stated in article.
[e] Range reflects 5th to 95th percentiles.
[f] 10th to 90th percentile.

serum samples in this study. Contrary to the above-mentioned studies, no significant age- or sex-related differences were found in a study by Wiesmann et al. *(52)*, who selected 200 healthy blood donors whom they divided equally in 5 age bands each of approximately 9 years, spanning 18 to 65 years (Table 1).

These seemingly opposing findings between studies may be a function of whether or not children are included in the study cohort, because evidence suggests that the largest influence of age occurs early in life. Furthermore, the heterogeneity of normal cohorts selected to establish reference intervals is challenging because

much of the interindividual difference cannot be accounted for, and sample sizes often are not large enough per age group to adequately compensate for this variability. The selection of so-called "normal" samples is also subject to interpretation—in some studies, study participants have been chosen who have symptoms that could indicate neurological disease and warrant invasive procedures such as a lumbar puncture, whereas for other studies participants have been selected who are completely healthy individuals. The characteristics of study populations are thus important considerations when evaluating the role of S100B or any biomarker in conditions of pathology, defining reference values, or matching controls and cases.

Studies in CNS Infections

EXPERIMENTAL STUDIES

Bertsch et al. *(41)* measured serum S100B in a mouse model infected with CNS *Candida albicans*. They found that concentrations were significantly increased in infected mice relative to control mice and peaked within 1 day postinfection, followed by a subsequent decline. The kinetics of S100B appear to reflect the infective process whereby pathogenic entry into the CNS leads to an immediate inflammatory response, cell destruction, and BBB breakdown. When the infection is contained, tissue damage is halted and S100B concentrations return to baseline. In a sheep model, Garnier et al. found that sheep fetuses exposed to infection in utero developed white matter injury. The degrees of injury and mortality were positively associated with S100B concentrations in both the fetal and maternal circulation, suggesting the value of S100B in the early detection of fetal white matter injury *(53)*. Another experimental model reveals the role of S100B as a marker of hypoxic distress in fetal sheep. Increased plasma S100B was associated with markers of metabolic acidosis, redistribution of blood flow from the peripheries, and currently used indicators of perinatal hypoxia *(54)*.

CLINICAL STUDIES IN ADULTS

Infante et al. *(32)* examined S100B in the CSF of adult patients with various neurological pathologies, including meningitis, dementia, hydrocephalus, acute cerebral infarction, multiple sclerosis, motor neuron disease, and lymphatic leukemia. S100B was significantly increased in the meningitis, dementia, and acute cerebral infarction groups, with the highest concentrations associated with dementia. The authors suggest that S100B in acute limited injury may peak early and clear the system swiftly, in which case accurate assessment is highly dependent on the timing of sampling relative to the onset of injury. Long-term increases in S100B likely represent ongoing injury or the inflammatory activation of glial cells. Unden et al. *(3)* found higher serum S100B in adults with cerebral infections compared with extracerebral infections. Viral encephalitis patients demonstrated the highest concentrations, and bacterial meningitis patients recorded higher concentrations than those with viral meningitis, which is congruous with the established cellular injury associated with these diseases. However, some of their encephalitis and meningitis patients did not demonstrate increased values despite radiological and clinical abnormalities, suggesting that S100B may produce some false-negative results when used as a marker of brain injury. Considering that S100B is a brain-derived protein, this finding may reflect the fact that these investigators sampled blood rather than CSF. Further, in one of these encephalitis patients, sampling was done a few days later than when it was performed for the patients with increased concentrations; S100B has been shown to peak earlier in the disease process and so the peak may have been missed *(32, 55)*. The authors also suggest that S100B may demonstrate false-positive results: some cases with extracranial infection and no evidence of cerebral involvement had increased S100B. The exact extracranial sources of these increases in S100B are not known; however, in these instances S100B was mostly only marginally increased and did not reach concentrations as high as in encephalitis. In general, CNS infections were the predominant source of increased S100B.

CLINICAL STUDIES IN CHILDREN

Having examined reference values in infants, Spinella et al. *(37)* compared CSF S100B between healthy children and those with established meningitis and found the latter had significantly higher concentrations. Hamed et al. *(1)* examined concentrations of S100B and markers of oxidative stress and antioxidative activity in 40 children with bacterial meningitis. S100B was increased in both serum and CSF samples. These investigators contended that brain injury resulting from bacterial meningitis is evidenced by the increased intrathecal production of S100B and markers of oxidative activity, and that the concentrations of these markers are related to injury severity. A study of infants with bacterial meningitis *(56)* found increases in CSF S100B, with the highest values recorded in infants who developed encephalitis in addition to their meningitis. An ROC indicated that a CSF S100B concentration above 1.0 μg/L was diagnostic for the early detection of bacterial meningitis–encephalitis, with a sensitivity of 91% and a specificity of 82%, with an area under the curve of 0.92. S100B also surpassed standard monitoring techniques in identifying the development of encephalitis *(56)* (Table 2).

Table 2. S100B data from clinical studies (μg/L).

Cohort and reference	Age	Pathology	n	Specimen	Mean (SD)	Median	Range	Cases > controls[a]
Pediatric cohort								
Hamed et al. *(1)*	< 15 years	Controls	20	Serum	0.13 (0.01)		0.11–0.16	
	<15 years	Bacterial meningitis	40	Serum	0.22 (0.07)		0.13–0.57	√
				CSF	0.61 (0.03)		0.55–0.7	
Spinella et al. *(37)*	0.08–1.5 years	Controls	107	CSF		0.71	0.48–1.07	
		Meningitis	34	CSF		1.1	0.91–1.4	√
Gazzolo et al. *(56)*	38 weeks	Controls	44	CSF		0.16	0.11–0.33	
	38 weeks	Bacterial meningitis	44	CSF		1.34	0.84–1.78	√
Mixed cohort								
Infante et al. *(32)*[b]	1–79 years	Controls	22	CSF	0.88 (0.08)			
		Lymphocytic meningitis	19		2.51 (0.71)			√
		Bacterial–fungal meningitis	10		5.06 (2.43)			√
		Dementia	6		2.09 (0.62)			√
		Acute cerebral infarction	10		1.69 (0.26)			√
Adult cohort								
Unden et al. *(3)*[c]	15–84 years	Viral encephalitis	5	Serum	0.58			√
Lins et al. *(55)*	41 (13) years	Controls	13	Serum	0.05 (0.03)			
				CSF	1.22 (0.49)			
		Bacterial meningitis	11	Serum	>Controls			√
				CSF	>Controls			√
		Viral meningitis	13	Serum	<Controls			X
				CSF	<Controls			X
Mokuno et al. *(23)*[b]		Controls	18	CSF	0.33 (0.09)		0.16–0.52	
		Encephalitis	6		1.9 (1.8)		0.34–5.1	√
		Meningitis	24		0.6 (0.48)		0.06–2.4	√
		Cerebral infarction	10		1.4 (1.4)		0.12–4.1	√
		Parkinson disease	18		0.58 (0.23)		0.21–0.96	√
		Cervical spondylosis	9		0.51 (0.2)		0.24–0.84	√
		Acute poly(radiculo)neuritis	5		0.83 (0.62)		0.2–1.8	√
		Chronic poly(radiculo)neuritis	6		1.5 (2.3)		0.34–6.6	√

[a] √, Cases > controls at $P > 0.05$; X, case > controls at $P < 0.05$.
[b] Only pathologies with S100B concentrations significantly greater than those of controls included, further detail in the original reports.
[c] Several other pathologies included in the study had elevated S100B concentrations; however, no descriptive statistics are reported in the reference.

Neuron-Specific Enolase

NSE (γ-enolase) is a stable cell-specific isoenzyme of the glycolytic enzyme enolase, a dimer protein made up of α, γ, and β subunits. Although highly localized to neurons and neuroendocrine cells, NSE has also been found in erythrocytes, liver, smooth muscle, and lymphocytes; however, in the latter cases, it is often of the hybrid (αδ) enolase form and has low concentrations (<10 ng/mg) *(11, 15)*. In the brain, NSE is concentrated exclusively in the cytoplasm of neurons *(11)*. NSE demonstrates enzymatic activity and is a promising marker of general neuronal function. Reference intervals for serum concentrations range from 5 to 15 ng/mL. When neuronal membranes are injured, NSE easily diffuses into the extracellular space and CSF *(26)*. Consequently, increased concentrations of NSE in the CSF and serum have been identified in several cerebral pathologies, including encephalitis, cerebral infarction, Parkinson disease, amyotrophic lateral sclerosis, spinocerebellar degeneration, cervical spondylosis, polyradiculoneuritis, and TBI *(23, 57, 58)*. NSE is

Table 3. NSE reference intervals (μg/L).

Cohort and reference	Age group	n	Specimen	Mean (SD)	Median	Range[a]
Pediatric cohort						
Rodriguez-Nunez et al. *(60)*	1 month–13 years	37	CSF	1.5 (1.01)	1.5	0–4.8
Rodriguez-Nunez et al. *(65)*	1 month–13 years	160	CSF	1.5 (1.01)		
Mixed cohort						
Van Engelen et al. *(4)*[b]	1 year		CSF			2.2–10.2[c]
	20 years					2.7–12
	40 years					3.1–13.8
	60 years					3.8–16
Adult cohort						
Casmiro et al. *(61)*	62.7 (16.7) years	108	Serum	8.7 (3.9)[d]		
			CSF	17.3 (4.6)		
Nygaard et al. *(59)*	20–89 years	87	Serum	7.1 (3.6)		6.2–8.1[e]
			CSF	5.1 (1.6) (males) 4.1 (1.4) (females)		

[a] Range is the 25th-to-75th percentile unless otherwise stated.
[b] Sample sizes not stated in article.
[c] Range is the 5th-to-95th percentile.
[d] Based on data from 98 patients.
[e] 95% CI.

also increased in small cell lung cancer *(15)* and pediatric neuroblastoma *(11)*.

REFERENCE VALUES

Van Engelen et al. *(4)* sought to establish reference values for NSE, S100B, and myelin basic protein (MBP). They collected CSF samples in 79 control patients ranging from 1 to 60 years of age. Their findings suggested a significant increase with age, which was similar across all 3 markers, with a median increase of 1% per year. Possible explanations proposed for this finding relate to an increase in cell and myelin loss with age and/or a parallel reduction in CSF bulk flow. Nygaard et al. *(59)* studied CSF and serum NSE concentrations in healthy individuals ranging from 20 to 90 years of age. In the serum they found no differences based on sex and age, but in CSF there was a positive significant association with age, and males had higher values. The authors suggest that the increase in NSE with age may be attributed to increased NSE concentrations in the face of stable cell turnover, increased cell turnover with age, or an increased NSE half-life due to reduced CSF bulk flow. Sex dependency may be explained by similar mechanisms. In addition, this study revealed no correlation between the NSE concentrations in the 2 biological fluids. However, the association with age is not a consistent finding. In a pediatric study, Rodriguez-Nunez et al. *(60)* examined NSE in the CSF of 37 children aged 1 month to 13 years. Their findings did not suggest an association with age. Casmiro et al. *(61)* studied both CSF and serum in an exclusively adult population of 108 individuals and also found no significant association with age; however, their sample was skewed to patients over 60 years old. There were no sex-related associations in NSE concentrations and no correlations between serum and CSF NSE values were demonstrated *(61)* (Table 3).

These results, in conjunction with findings of Nygaard et al. *(59)*, suggest that serum cannot substitute for CSF and that serum NSE within the reference interval does not necessarily exclude the presence of CNS injury, although this requires validation in a sample of patients with CNS disease. Studies on reference intervals in NSE are subject to the same limitations and cautionary interpretation as for S100B.

Studies in CNS Infections

EXPERIMENTAL STUDIES

The authors are unaware of any studies of NSE concentrations in experimental models of CNS infection. However, a study of NSE in an ischemic rat model is relevant because ischemia is an important determinant of poor outcome in CNS infections. Hardemark et al. *(62)* correlated NSE concentrations in CSF with the development and size of infarcts in rats by sampling fluid before and after middle cerebral artery occlusion over several days. NSE peaked between 24 and 72 h

after occlusion, with a subsequent return to baseline over 6 days. Infarct size was significantly correlated with NSE concentrations and the area under the NSE concentration curve. These findings suggest that sequential analysis of CSF NSE reflects the development as well as size of infarcts. The temporal variation of NSE reflects the dynamic nature of the ischemic process, which may occur over several days and is subject to interindividual variation. The timing of sampling as the injury evolves is therefore an important consideration.

CLINICAL STUDIES IN ADULTS

Lima et al. *(26)* examined whether NSE concentrations correlate with severity of neurological impairment. They sampled serum and CSF in 51 adults: 11 with meningitis, 7 with encephalic injuries, 25 with neurocysticercosis, and 8 controls. They found no age or sex differences. NSE was increased only for the group with encephalic injuries, and only in CSF. It is worth noting that none of the meningitis group was neurologically compromised and so the degree of neurological injury is debatable. Song et al. *(63)* measured NSE in the CSF and serum of adult patients with tuberculous meningitis (TBM) ($n = 15$) and aseptic meningitis ($n = 28$) in comparison with controls. Although serum and CSF NSE concentrations were not significantly different across the 3 groups, the CSF/serum NSE ratio was significantly higher in the TBM group. This ratio was significantly correlated with the diagnosis of TBM in univariate and multivariate analysis, demonstrating its potential as a diagnostic marker. The sample size of this study was small and provides a limited picture of NSE concentrations in meningitis.

CLINICAL STUDIES IN CHILDREN

There is a dearth of literature available regarding biomarkers for brain injury in children, with most of the research in TBI *(19, 57, 58, 64)*. Rodriguez-Nunez et al. *(65)* examined CSF markers of cell hypoxia and NSE to discern the utility of these markers in the differential diagnosis of meningitis. They examined 160 control children and 100 children with bacterial, viral, or tuberculosis meningitis and found that NSE concentrations in bacterial meningitis and TBM cases were not significantly higher than those in controls, did not differ on the basis of sex, and were not different between meningitis groups. Only the viral meningitis group had significantly increased NSE; however, this was attributed to 9 cases with mumps etiology, in whom NSE concentrations were highest overall. Two limitations of this study were, first, their TBM group was very small, comprising only 0.9% of the meningitis cases, and second, the CSF samples were collected very early in the disease course (1–16 h after the onset of symptoms). Experimental work in animal models suggested that NSE tended to increase beyond 24 h postinjury *(62)*; therefore, the samples may have been taken when hypoxia had not been sufficiently prolonged or intense to induce cell death and cause increases in NSE. In a study of 20 comatose children suffering from acute encephalitis, acute encephalopathy, and Reye syndrome, Nara et al. *(66)* found that both CSF and serum NSE concentrations were significantly increased in these children relative to concentrations in controls. NSE was associated with neurological compromise and showed higher initial values and subsequent increases in patients with more severe brain damage and poorer outcome. Their findings support the variability of NSE concentrations at different disease stages and between patients. Contrary to other studies, in this study the authors found a good correlation between serum and CSF NSE (Table 4).

Combining Biomarkers

Lins et al. *(55)* performed sequential paired CSF and serum measurements of S100B and NSE in patients with bacterial and viral meningitis. Results from 32 adult patients revealed significantly increased serum and CSF S100B in patients with bacterial meningitis only. Increased S100B was associated with lesions on computed tomography or MRI and higher CSF/serum albumin ratios. In most patients, S100B decreased after the initial samples, except in a patient with TBM in whom biomarker concentrations in CSF and serum continued to climb, peaking by day 27. NSE was not significantly increased in this study. Mokuno et al. *(23)* examined CSF S100B and NSE in patients with a range of neurological pathologies, including encephalitis, meningitis, cerebral infarction, Parkinson disease, sclerosis, and others. NSE and S100B were increased in several of these conditions, but not always simultaneously, suggesting that their presence may reflect the kind of tissue damage, i.e., glial vs neuronal. Concentrations were highest in encephalitis and cerebral infarction, and S100B concentrations normalized earlier than NSE concentrations.

Other Potential Biomarkers

Although studies on S100B and NSE dominate the literature, work is also being done on other biomarkers. In a case series of 3 patients with cerebral infective disease, Van Engelen et al. *(4)* examined CSF concentrations of myelin basic protein (MBP), a marker of myelin breakdown, as well as S100B and NSE. They found that concentrations of all 3 biomarkers were increased and that their relative values were in line with the nature of the injury; in patients with demyelinating illnesses, MBP concentrations were higher than S100B and NSE. Tsukahara et al. *(67)* examined CSF S100B,

Table 4. NSE data from clinical studies (μg/L).

Cohort and reference	Age	Pathology	n	Specimen	Mean (SD)	Median	Range	Cases > controls[a]
Pediatric cohort								
Rodriguez-Nunez et al. *(65)*	1–168 months	Controls	160	CSF	1.52 (1.01)			
		Viral meningitis	46		2.87 (1.21)			√
		Bacterial meningitis	45		2.47 (1.59)			X
		Tuberculous meningitis	9		2.25 (0.49)			X
Mixed cohort								
Lima et al. *(26)*	13–82 years	Controls	8	Serum	4.7 (2.3)	4.9		
				CSF	6.3 (3.6)	6.1		
		Meningitis	11	Serum	5.8 (4.6)	5.4		X
				CSF	4.9 (4.6)	4.4		X
		Neurocysticerosis	25	Serum	6.6 (3.4)	7.1		X
				CSF	3.9 (3.2)	3		X
		Encephalitis	7	Serum	20.9 (19.2)	9.2		√
				CSF	35.8 (39.2)	29.7		√
Adult cohort								
Lins et al. *(55)*	41 (13) years	Controls	13	Serum	5.82 (1.75)			
				CSF	6.78 (2.1)			
		Bacterial meningitis	11	Serum	5.1 (1.3)			X
				CSF	8.38 (3.7)			X
		Viral meningitis	13	Serum	5.73 (3.7)			X
				CSF	8.23 (3.4)			X
Mokuno et al. *(23)*[b]		Controls	18	CSF	4.5 (1.2)		1.3–7.2	
		Encephalitis	6		11.3 (12.9)		3.4–40	√
		Amyotrophic lateral sclerosis	10		6 (2.1)		2.5–10	√
		Cerebral infarction	10		9.9 (6.2)		3.3–25	√
		Parkinson disease	18		7 (3.6)		0.8–16	√
		Cervical spondylosis	9		8.7 (3.4)		4.1–14.4	√
		Acute poly(radiculo)neuritis	5		6.2 (2.3)		4–10	√
		Chronic poly(radiculo)neuritis	6		2.5 (0.9)		1.2–4.2	√
		Spinocerebellar degeneration	10		7.2 (2.5)		3.2–10.8	√
Song et al. *(63)*	Adults	Control	37	Serum	10.42 (6.06)			
				CSF	9.46 (5.76)			
		Tuberculous meningitis	15	Serum	8 (2.17)			X
				CSF	12.35 (3.52)			X
		Aseptic meningitis	28	Serum	9.57 (7.16)			X
				CSF	9.79 (6.1)			X

[a] Range is the 25th-to-75th percentile unless otherwise stated. √, cases > controls at $P > 0.05$; X, case>controls $P < 0.05$.
[b] Only pathologies with S100B concentrations significantly greater than those of controls included in table, further detail in original article.

glial fibrillary acidic protein (GFAP) (a marker of glial injury), and tau protein (a microtubule-associated protein found in neuronal axons) in children with acute encephalitis/encephalopathy. CSF biomarker concentrations were increased only in patients with poor outcome, and S100B had the best predictive accuracy with respect to outcome. GFAP showed poor sensitivity (40%) but was very specific (100%), whereas tau protein had a sensitivity of 70% and specificity of 85%. Combining several markers allowed for the best

predictive value for outcome. In a cohort of patients with varicella zoster virus CNS infections, Grahn et al. *(68)* measured CSF S100B, GFAP, and neurofilament protein light chain (NFL), a potential marker of neuronal injury. NFL showed the highest and most prolonged increases, followed by GFAP, whereas S100B concentrations were within reference intervals. This suggests that damage may have involved astrogliosis while the astrocyte cell membrane remained intact, and that persistent or secondary injury may be responsible for ongoing NFL elevation. A review by Bonneh-Barkay et al. *(8)* summarizes research into biomarkers for encephalitis in adults; CSF NSE, NFL, GFAP, S100B, a variety of proinflammatory cytokines, and soluble Fas (an apoptotic marker) were identified in herpes encephalitis. The cytokines interleukin-6 and tumor necrosis factor-α together with metabolites showed promise in influenza-associated encephalitis. CSF S100B and NFL were investigated in West Nile virus encephalitis. Work on HIV encephalitis has included the examination of CSF HIV-1 RNA, factors associated with immune activation, like neopterin, and markers of inflammation.

Advances in genomics, transcriptomics, proteomics, and metabolomics offer opportunities for investigating various molecular aspects of the host–pathogen interaction in CNS infections. These include pathogen-specific molecular or metabolic signatures, pathogen-directed alteration to host gene expression patterns, genetic susceptibility to infections, interindividual genetic variation in response to pathogen exposure, and the association between gene expression profiles and disease outcome. These technologies therefore have the potential to identify biomarkers that could accurately diagnose the disease as well as the offending pathogen, allow early selection of appropriate treatment, identify patients at greater risk of severe disease and poor outcome, and allow treatment tailored to both host and pathogen characteristics. This field, however, is still in a fledging state, particularly with regard to CNS infections *(5)*. In the field of proteomics, Goonetilleke et al. *(69)* used 2-dimensional gel electrophoresis to study the CSF protein profile of patients with pneumococcal meningitis. Increases in various proteins were associated with inflammation, cellular damage, and metabolic derangement in nonsurvivors and could provide insight into novel biomarkers and drug targets. In a proteomics study on the CSF of TBM patients, Kataria et al. *(70)* found 18 differentially expressed proteins. Upregulated proteins of human origin included arachidonate 5-lipoxygenase, which contributes to the pathophysiology of TB by weakening the host's immune responses; GFAP, a marker of tissue damage; and antithrombin-III, which indicates the involvement of coagulation pathways. Downregulated human proteins included apolipoprotein E and A-1, which are important for their antiinflammatory properties and for neural growth and repair and immune regulation, and transthyretin, which aids in reducing brain edema, neuronal death, and inflammation. Mycobacterial proteins identified in the CSF could present diagnostic markers for swift detection of the causative agent in patients with meningitis. Metabolomics is a high-throughput technology that allows for a broad unbiased analysis of the full complement of metabolites present in biological samples. It is increasingly being used in disease diagnostics, therapeutics, and drug development because it provides insight into the combined effects of metabolites from the infecting organism, inflammatory cells, and cerebral cellular responses to pathogens and therapeutics *(71)*. In a study on patients with meningitis and ventriculitis, Coen et al. *(72)* demonstrated that metabolomics of the CSF could rapidly diagnose these infections and showed improvements in the performance of routine CSF cell counts and chemistry assays. Subramanian et al. *(73)* developed an expert system to aid in the differential diagnosis of meningitis using a unique combination of clinical features and metabolic fingerprints.

Future Work

More in-depth studies looking at broader panels of markers are required. A combination of neurological markers may also be useful in tracking injuries of various tissue types and may mitigate the influence of extracranial sources. Methods combining inflammatory and tissue injury markers have demonstrated improved prognostic power in TBI *(20)* and may prove beneficial in CNS infections as well. Few of the available studies in CNS infections examined biomarkers in relation to outcomes. Several studies have confirmed significant associations between biomarker concentrations and radiological and clinical outcome in TBI *(21, 74, 75)*; because these are the current tools available it is important to ascertain the strength of the associations with biomarkers in infectious injury.

Conclusion

Despite the limited number of studies of biomarkers in CNS infections, the literature available suggests that in both adults and children biomarkers of neurological injury have the potential to answer several important questions: (*a*) when presenting symptoms and history are nonspecific, is there CNS involvement?; (*b*) what is the nature of the pathological process?; (*c*) what is the likely causative pathogen?; (*d*) which cells of the CNS are affected?; (*e*) how severe is the injury?; and (*f*) is the disease process responding to intervention? Biomarkers of neurological injury carry promise as markers of

diagnosis as well as prognosis and may serve as surrogate end points for determining the response to novel interventions. Further studies on biomarkers in this field will add to our understanding of their utility. When conducting biomarker studies it is important to consider limitations presented by heterogeneity in the study cohort, the relative value of CSF vs serum, and the choice of testing platform.

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