Cerebrospinal fluid and blood biomarkers of status epilepticus

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Summary

Status epilepticus is a condition resulting either from the failure of the mechanisms responsible for seizure termination or from the initiation of mechanisms which lead to abnormally prolonged seizures and require urgent administration of antiepileptic drugs. Refractory status epilepticus requires anesthetic drugs and may lead to brain injury with molecular and cellular alterations (e.g. inflammation, neuronal and astroglial injury) that could induce neurological sequels and further development of epilepsy. Outcome scores based on demographic, clinical and EEG condition are available, allowing to predict the risk of mortality, but the severity of brain injury in survivors is poorly evaluated. New biomarkers are needed in order to predict with higher accuracy the outcome of patients admitted with status in an intensive care unit.

Here, we summarize the findings of studies from patients and animal models of status epilepticus. Specific protein markers can be detected in the cerebrospinal fluid and the blood. One of the first described markers of neuronal death is the neuron-specific enolase. Gliosis resulting from inflammatory responses after status can be detected through the increase of S100-beta, or some cytokines, like the High Mobility Group Box 1. Other proteins, like progranulin may reflect the neuroprotective mechanisms resulting from the brain adaptation to excitotoxicity. These new biomarkers aim to prospectively identify the severity and development of disability, and subsequent epilepsy of patients suffering from status.

We discuss the advantages and disadvantages of each biomarker, by evaluating their brain specificity, stability in the fluids and sensitivity to external interferences, such as haemolysis. Finally, we emphasize the need of further development and validation of such biomarkers in order to better assess patients with severe status epilepticus.

Abbreviations: BBB = blood-brain barrier; GFAP = glial fibrillary acidic protein; HMGB1 = High Mobility Group Box 1; NSE = neuron specific enolase; PGRN = progranulin; SE = status epilepticus

Key words: epilepsy, prognosis, critical care, neuronal injury, inflammation

Introduction

Epilepsy is one of the most common neurological disorders which affects approximately 0.7% of the population ¹. Status epilepticus (SE), is a condition resulting either from the failure of the mechanisms responsible for seizure termination or from the initiation of mechanisms which lead to abnormally prolonged seizures ². Most SE can be treated by adequate antiepileptic drugs, and such patients generally recover well. Nevertheless, in around 25% of cases, SE is refractory and requires anesthetic drugs ³. For these patients, SE may lead to brain injury with cellular and molecular alterations (e.g. inflammation, or neuronal and astroglial injury) that could induce subsequent irreversible neurological sequels (Fig. 1), further development of epilepsy, with a mortality rate ranging from 7% to 39%. ⁴. Difference in mortality depends on the semiology of SE (increased mortality in non-convulsive SE with coma), the level of consciousness (increased case fatality rate in patients with impaired consciousness), and age (increased mortality in subjects over 65 years) ^{4,5}.

New biomarkers combined with clinical data, EEG (Fig. 2) and MRI (Fig. 1), may help clinicians to predict SE outcome with more accuracy ^{6,7}. Cerebrospinal fluid (CSF) biomarkers have already been established in neurological diseases such as Alzheimer's disease, Parkinson's disease, multiple sclerosis or in the traumatic brain ^{8–11}. In SE, even if CSF biomarkers have been proposed, none have been yet validated in clinical use to diagnose SE or predict its clinical outcome.

In this review, we provide an overview of the current research on promising biomarkers of SE in CSF and blood (Table 1, Fig. 3). We present arguments for further development and validation of such biomarkers, and for their use in assessing patients with SE.

How biomarkers can help management of SE?

There is a large number of possible clinical presentations of SE (i.e. focal SE, absence SE, generalized convulsive SE, myoclonic SE, non convulsive SE) ¹². The diagnosis of convulsive SE relies mainly on clinical assessment. On the other hand, the diagnosis of non-convulsive SE relies on EEG recording (Fig. 2) which requires specialized clinical staff and equipment that are not available to all medical units at all times. While clinical semiology and EEG are the best features for a positive diagnosis of SE, non-EEG biomarkers would be of great help, in particular for the diagnosis of the non-convulsive SE.

Clinical SE prognosis factors, such as generalized convulsive status, patients of advanced age, and long duration of SE have been correlated with higher mortality rate ⁴. But the most important outcome factor is the underlying aetiology: patients with post-anoxic SE, acute brain injury have a higher mortality rate than patients with a SE related to antiepileptic drug withdrawal. Nevertheless, consequences of SE from a single type of aetiology are heterogenous. Outcome scores based on demographic, clinical and EEG condition are available ¹³. However, they predict the bad outcome (i.e. death) but the severity of brain injury in survivors is poorly evaluated. New biomarkers are needed to predict with a higher accuracy the outcome of patients admitted with SE in an intensive care unit. Even if some biochemical biomarkers are not specific to SE, we assume that they can be combined with clinical and paraclinical data (EEG and MRI) to help clinicians predict SE outcome.

Biomarkers able to identify SE aetiology may be very useful in clinical practice. There are now guidelines to identify the aetiology of SE in acute phase ¹⁴. The Status Epilepticus Etiology Identification Tool (SEIT) is based on semiology, head computer tomography scan, and on first level blood tests (blood glucose, basic metabolic panel, anti-epileptic drug levels if appropriate,...). According to these first results, this tool aims to propose more specific blood or CSF tests ¹⁴. We can speculate on our future ability to isolate specific biomarkers of acute causes of SE. Similarly, we may identify biomarkers such as cytokines levels or specific interleukines, that could suggest infectious or auto-immune SE.

Biomarkers may also be used to identify specific etiologies requiring individualized treatments. Thereby, a recent use of IL6 monoclonal antibodies has been specifically proposed in NORSE and IL-1 receptor antagonist in FIRES ¹⁵.

Physiologic responses to status epilepticus and late consequences

A good fluid biomarker is a molecule: (i) whose measurements in fluid provides high sensitivity and high specificity tests; (ii) whose biochemical assessment is easy, quick and reliable and (iii) which is stable in the body fluids and demonstrates links to molecular and cellular alterations that occur in SE.

During SE, physiologic responses occur first and then pathological late consequences can lead to neuronal death and subsequent epilepsy.

During convulsive SE, some physiologic changes occur outside of the brain; many of them may worsen the acute brain injury. They include: (i) hyperthermia ¹⁶; (ii) immediate and marked increase in plasma catecholamine concentrations, that may provoke increased cerebral blood flow ¹⁷; (iii) hypoglycemia; (iv) marked and rapidly developing acidosis, which could be beneficial or harmful for the brain as the acidosis is respectively modest or significant ¹⁸.

Our understanding of the late consequences of SE and epileptogenic process comes largely from animal models of induced SE. Epileptogenesis refers to the phenomenon in which an initial brain insult (e.g. traumatic brain injury, stroke, or SE) triggers a cascade of molecular and cellular changes that eventually culminate in the occurrence of spontaneous seizures. Five leading changes can be identified after a SE. (i) One major change is the neuronal death. The origin of this acute neurodegeneration mainly comes from excitotoxic processes. It was described in the hippocampus, amygdala as well as in many extratemporal areas including the thalamus¹⁹. The therapies used to reduce neurodegeneration decrease the behavioral consequences after SE in rats ²⁰. Neuronal loss can be evaluated by the blood concentration of specific proteins such as neuronal markers ²¹. (ii) Increased neurogenesis could be detected within few days and remained up to four weeks after the onset of SE ²². This process may reflect the brain adaptation to the neuronal death ²³, and can be evaluated by the levels of mediators involved in neuronal growth factor, such as progranulin ²⁴. Nevertheless, neurogenesis is limited to some brain areas, especially the hippocampus. (iii) Gliosis results from inflammatory responses ^{25,26}. An increased expression of glial cell markers (glial fibrillary acidic protein) and proteins secreted by glial cells (e.g. cytokines, chemokines) was reported, as a consequence of cytokines release during excitotoxic processes and glial cell activation related to cleaning processes after neuronal death. Gliosis may modify ionic homeostasis and further enhance neuronal excitability. (iv) Blood-brain barrier breakdown may contribute to the development of epileptogenesis *via* a direct mechanism (influx of potassium) or *via* the leakage of serum proteins in the brain causing glial cells activation as well as inflammation and impaired potassium buffering ²⁷. (v) Axonal injury can be evaluated by measuring tau protein in the cerebrospinal fluid.

Potential cerebrospinal fluid and blood biomarkers of status epilepticus

Cerebrospinal fluid (CSF) is a source of promising biomarkers. CSF is in close contact with the extracellular space of the brain compared to the plasma or the serum, and its composition closely reflects biochemical changes that occur in the brain ^{8,9}. CSF biomarkers have already been established in neurological diseases such as Alzheimer's disease, Parkinson's disease, multiple sclerosis or in the traumatic brain injury ⁸⁻¹¹.

In SE, even if CSF biomarkers have been proposed, none has yet been validated for clinical use. Some proteins that are highly expressed in the brain (e.g. S100-B, NSE, and cytokines) can be detected in the serum, even if in low concentrations. It will be very useful to benefit from blood biomarkers of SE because a lumbar puncture is invasive, not systematically performed in routine clinical care for patients in SE, and can not be easily repeated, for days- or weeks-long kinetic studies and patients follow-up.

Here we present an overview of biomarkers relating to the pathophysiology of SE.

Acidosis

A marked and rapidly developing acidosis is noted in patients with SE and in animal models of SE. In humans, a study conducted in twenty-nine adult patients with generalized SE of various etiologies showed an increase of CSF lactate for all SE patients with a mean at 3.74 ± 0.31 mM compared to control subjects (1.60 ± 0.10 mM) ²⁸. This study suggested that the magnitude of lactate elevation may serve as a predictive indicator of morbidity or mortality because patients with poor outcome had significantly higher CSF lactate levels (5.36 ± 0.58 mM) than those from patients who recovered well (3.01 ± 0.22 mM). However, CSF lactate elevation is common after acute brain injury and might not be specific to convulsive SE ²⁹.

Acidosis was also observed in a rat model of SE induced by injection of bicuculline, the pH falling from 7.41 ± 0.06 to 6.91 ± 0.48 five minutes after induction of epileptic seizures ³⁰.

However, there are other sources of acidosis of which muscle contraction. Although lactate is elevated after muscle contraction which is obviously potentially increased after convulsive SE, acidosis is not specifically associated with SE. Nevertheless the correlation between CSF lactate and outcome is promising and acidosis might provide a relevant biomarker of SE.

Acute neuronal injury

Markers of neuronal injury like Neuron Specific Enolase (NSE), also called enolase-2, could provide an estimate of the degree of brain injury in SE. Enolase is an enzyme expressed in the cytoplasm of all tissues able to perform glycolysis. It catalyzes the conversion of 2-phospho-D-glycerate to phosphoenolpyruvate, and represents a key enzyme of anaerobic glycolysis (a reaction that drives glucose to lactate). Many isoenzymes have been described, depending of which subunits (alpha, beta, gamma) make up the dimers. In the nervous system, glial cells express the $\alpha\alpha$ isoform. Neurons express the NSE, a very stable homodimer of γ subunits with a half-life of around 24 hours. It is found in the central and the peripheral nervous system or neuroendocrine tissue, particularly in cells from the APUD system (Amine Precursor Uptake Decarboxylation). Its assay is performed using a monoclonal antibody specific of the γ subunit.

NSE levels were first studied in relation to focal or generalized seizures ³¹. Patients with frequent seizures or pharmaco-resistant seizures showed elevated levels of NSE in both serum and CSF ³². However, there are conflicting findings for correlation between NSE levels and seizure duration ^{33,34}.

An increase of CSF concentrations of NSE was first reported in nine of eleven patients with cryptogenic/remote symptomatic SE (without any acute neurologic insult for more than one

month), compared to twelve control subjects. The mean of CSF-NSE concentrations was higher in SE patients than in control patients (30.8 ± 18.33 versus 10.76 ± 3.08 ng/mL) ³⁵. In a prospective study monitoring serum NSE in nineteen patients with sustained SE including eleven without any acute neurologic insult for more than one month, NSE dosages were performed at 24, 48, 72 hours, and seven days after the diagnosis of SE ³⁶. The mean peak of serum NSE for the entire group (24.87 ng/mL) and for the eleven patients without an acute neurologic insult (15.44 ng/mL), at 24 and 48 hours after the onset of SE, were increased in comparison to the levels for normal controls (5.36 ng/mL) and controls with epilepsy (4.61 ng/mL). NSE was normalized at seven days after SE. Moreover, outcome was highly correlated with the peak of serum NSE: serum NSE levels were inversely correlated with the Glasgow Outcome Scale at one week. NSE levels also correlated with the duration of SE: in patients with normal NSE levels at the onset of the SE, SE was significantly shorter than in patients with higher NSE levels ³⁶. In another study, serum NSE levels were measured in four subtypes of SE: complex partial SE, absence SE, generalized convulsive SE and acute symptomatic myoclonic SE (of which one third is secondary to anoxia) ³⁷. NSE was significantly elevated in all four subtypes compared to control subjects (5.02 ng/mL), and NSE levels were higher in complex partial SE (23.88 ng/mL) and in acute symptomatic myoclonic SE (37.83 ng/mL). No significant modification in the levels of NSE were found in patients with remote symptomatic SE (i.e. patients without any acute neurologic insult for more than one month, or who had chronic epilepsy or drug withdrawal) when compared with acute symptomatic SE (i.e. patients with any neurologic insult within one month including anoxia) ³⁷. The highest level of NSE in acute symptomatic myoclonic SE was correlated with the worst outcome, in accordance to the Glasgow Outcome Scale.

Similar results were observed in rats with a SE induced by lithium pilocarpine. In this model, serum NSE levels increased from 5.4 ± 0.4 ng/mL in the control condition to 30.4 ± 1.3

ng/mL after SE. Elevation of serum NSE levels after SE correlated with overall histologic evidence for brain damage ²¹.

Elevated levels of NSE in the serum may be explained by an increased permeability of the blood-brain barrier ³⁵. NSE has a quite good neuronal specificity - except in tumoral context - and seems to be correlated with the duration and the outcome of SE, two critical requirements for a valid prognosis biomarker. Nevertheless, two antiepileptic drugs commonly used in clinical practice (i.e. carbamazepine and oxcarbazepine) could decrease serum NSE levels ³⁸. Moreover, NSE is highly sensitive to haemolysis in peripheral blood as well as in CSF. Indeed, NSE levels are markedly increased by *in vitro* lysis of erythrocytes and thrombocytes, and an artificial increase of NSE level could be seen since the haemoglobin concentration is up to 47 mg per dL ³⁹. An adapted pre-analytical approach is needed to avoid haemolysis for a good monitoring of this biomarker.

Therefore, despite good characteristics (good neuronal specificity, stability in the body fluid, links to cellular alterations that occur in SE (i.e. neuronal death) and a good correlation with the duration and outcome of SE), NSE is not currently used as a validated biomarker of SE.

Blood-brain barrier integrity

The blood-brain barrier (BBB) is the most important vascular barrier of the central nervous system (CNS). It is constituted by a specialized vascular endothelium that interacts with astrocytes, neurons and pericytes and limits penetration of a variety of substances from the blood into the brain. An increased number of spontaneous seizures in pilocarpine-exposed rats was observed in rats with greater BBB dysfunction, suggesting a potential direct role of BBB dysfunction in epileptogenesis ⁴⁰. BBB dysfunction can be measured by the CSF/serum albumin ratio ⁴¹. Albumin is mainly synthesized in the liver; therefore, most of albumin in CSF is derived from blood via passage across the BBB. An increase of the CSF/serum

albumin ratio, indicating BBB damage, is found in patients with various CNS disorders, such as inflammatory diseases, brain tumours or traumatic brain injury.

An increase of the CSF/serum albumin ratio was reported in eleven patients with SE (33.4×10^{-3}) compared to twenty control patients (4.79×10^{-3})³⁵. Higher CSF/serum albumin ratio were found for thirty-one patients with generalized tonic-clonic seizures compared to twenty-five patients without seizure (8.4 ± 2.6 versus 4.7 ± 1.4)⁴².

Acute astroglial injury

The leakage of serum proteins in the brain due to a BBB dysfunction could lead to a glial activation and inflammation. In SE, increased expression of markers of glial cells (glial fibrillary acidic protein) and proteins produced by glial cells (e.g. cytokines, chemokines, and protein S100-beta also called S100-B) have been reported.

The S100 proteins are a family of calcium-binding proteins with both intracellular and extracellular functions such as the maintenance of cytoskeleton structure, the intracellular communication, the regulation of the cell cycle and energy metabolism and a role in nerve cells growth. The S100 proteins are composed of two subunits alpha and beta, forming dimeric structures, i.e. $\alpha\alpha$, $\alpha\beta$ or $\beta\beta$. The protein S100-B has at least one beta subunit. S100-B was previously thought to be specific to astrocytes and Schwann cells but S100-B was also detected, albeit at low concentrations, in extracerebral cell types (e.g. chondrocytes, adipocytes, melanocytes). S100-B has a renal elimination and a half-life in blood of around 1.5 hours. At nanomolar concentrations, S100-B can enhance survival of neurons during development and stimulate neurite outgrowth in cortical neurons⁴³. In comparison, micromolar levels of S100-B have toxic effects by inducing apoptosis and stimulating the expression of proinflammatory cytokines⁴⁴. S100-B was proposed as a biomarker of neurological disorders thanks to its expression in brain and CSF, its low concentrations in

serum of normal subjects, its possible extravasation in case of BBB disorder and its release from cellular lysis ⁴⁵. An increase of CSF and serum S100-B levels was reported in acute brain injury such as in traumatic brain injury in proportionate terms with the severity of injury ⁴⁶.

S100-B was studied in isolated seizures. We conducted a study in order to evaluate the performance of S100-B and copeptin in addition to clinical variables, in predicting the outcome of patients hospitalized in emergency department following seizures ⁴⁷. S100-B serum levels were shown to be higher in patients who experienced unfavourable outcome (death, hospitalization, seizure recurrence, rehospitalization or a return to the emergency department within seven days) in comparison to other patients. Nevertheless, this biomarker did not improve the prediction of poor outcome following seizures ⁴⁷. To the best of our knowledge, no other study evaluated S100-B as a biomarker in SE patients.

The dynamic of astroglial alterations was studied in the lithium-pilocarpine rat model of SE. S100-B CSF levels were increased from the first day (during SE) to the late period marked by the occurrence of spontaneous seizures, suggesting astroglial activation after the onset of SE ⁴⁸. In the serum, S100-B levels were decreased the day after the lithium-pilocarpine induced SE and increased at 14 days, when compared with levels after sham treatment. The increase in serum S100-B levels suggests astroglial activation and long-term changes in BBB.

S100-B is a potential biomarker for SE: it is expressed in brain and CSF, can be released in the blood in case of BBB alterations like in SE and is increased in a rat model of SE and in patients with seizures. Nevertheless, it still has to be evaluated in patients with SE.

Glial fibrillary acidic protein (GFAP) is a CNS-specific intermediate filament that is exclusively expressed in astrocytes located in the white matter. In the CNS, GFAP is of paramount importance for the structural organisation of astrocytes, the communication

between astrocytes and some neurons types, including Purkinje cells, and in maintaining the integrity of the BBB.

Elevated CSF GFAP levels were shown in children with seizures as compared with controls⁴⁹. In addition, seizure duration was positively correlated with CSF GFAP levels along with SE severity. On the other hand, the aetiologies of the seizures did not seem to affect GFAP level. To the best of our knowledge, no other study in children or adults was conducted. In blood, GFAP might be a better biomarker of SE than S100-B because extracerebral production of GFAP was never detected. Immunohistochemistry studies in animal models of SE have shown increased progressing GFAP staining after SE in hippocampus, substantia nigra and cortical areas⁵⁰.

To conclude, based on the available studies conducted in animal model of SE and in patients with epilepsy, S100-B and GFAP have the potential to be relevant biomarkers of SE due to their expression in brain and CSF, their ability to be released in the blood and their increase after SE. Nevertheless, additional studies on a large cohort of patients with SE with longitudinal follow-up are necessary to better evaluate the capacity of these biomarkers to identify patients with SE and to predict their clinical outcome.

Neuroinflammation

There is increasing evidence for the role of brain inflammation in epilepsy. Glial cells activation secondary to the BBB breakdown or neuronal death can induce neuroinflammation and can be followed by the increase of cytokines/chemokines in CSF and in blood of patients with SE. Cytokines such as IL-1 β , IL-6, CRP and the High Mobility Group Box 1 (HMGB1) were measured to investigate their potential biomarker interest in epilepsy.

HMGB1 fulfils many criteria for a good fluid biomarker, including a good stability in blood. Higher HMGB1 staining was found in mice astrocytes after intrahippocampal injection of kainic acid and in humans epileptic tissues obtained from hippocampal sclerosis surgery ⁵¹. In addition, antagonists of HMGB1 increase delay before the first spontaneous seizure and decrease seizure recurrence ⁵¹. It has to be evaluated in human body fluids too.

Conflicting results were observed for IL-1 β . Interleukin-1 receptor (IL-1R) and its ligand, the pro-inflammatory cytokine IL-1 β are often expressed in association with BBB breakdown and neuronal damage suggesting a key role for the BBB in the brain inflammation observed in both mice and humans with epilepsy ²⁵. An increase of IL-1ra, the interleukin-1 receptor antagonist, and a decrease of IL-1 β levels were reported in the CSF of eight patients after prolonged focal or recurrent tonic-clonic seizures in comparison to sixteen control subjects ⁵². Increased ⁵³, or decreased blood IL-1ra levels were also reported in patients after seizures ⁵⁴. Furthermore, a study conducted in 85 children with generalized tonic-clonic seizures reported a positive correlation between IL-1 β and NSE levels in CSF ⁵⁵. To the best of our knowledge, no other study evaluated IL-1 β as a biomarker in SE patients.

In an animal model of SE, an induction of IL-1R and IL-1 β was shown in brain cells following SE ^{56,57}. For instance, a rapid and persistent increase of IL-1 β mRNA was observed in the hippocampus of a rat model of SE. IL-1 β is a less stable molecule explaining why time that elapses between the seizure occurrence and the samples could play a major role in the variable results described so far.

IL-6 seems to be a good fluid biomarker showing an increase of blood levels described in all studies conducted in patients with epilepsy and in animal models of SE ^{53,58}. Increased CSF IL-6 was also observed after recurrent generalized tonic-clonic seizures in patients ⁵⁸. However, there is a lack of studies conducted in patients regarding the cytokines levels in blood or CSF during SE.

An acute inflammatory response occurs within the SE. Nevertheless, to the best of our knowledge, the disturbances in cytokines levels and the correlation between the blood cytokines or HMGB1 levels and the patients outcome after SE was not investigated.

Acute axonal injury

The two best-established CSF biomarkers of axonal injury are tau protein, a phosphorylated microtubule-associated protein, and neurofilament light polypeptide (NFL).

Only few studies evaluated the potential of tau protein as a prognosis biomarker of seizure-related neuronal damage ⁵⁹. CSF levels of tau protein were evaluated in patients with SE and correlated with prognosis ⁶⁰. Out of twenty-eight patients with SE, without acute brain injury, CNS infection or neurodegenerative disorders, CSF tau levels were increased in fourteen patients. In addition, higher CSF tau levels were measured in patients who developed a refractory SE compared to patients with SE controlled by antiepileptic drugs. Moreover, CSF tau levels were positively correlated with SE duration and higher risk of sequels and subsequent epilepsy. Nevertheless, CSF tau levels were significantly higher in patients who were treated by propofol, which is known to induce tau phosphorylation ⁶¹. The marked increase of tau protein in refractory and super-refractory SE cases, and the capacity of tau protein to prospectively identify patients with status severity, development of disability and subsequent epilepsy deserve future verifications.

Neurofilaments are composed of neuron-specific intermediate filaments. Each filament consists of one light subunit (NFL) and a medium subunit (NFM) or a heavy subunit (NFH). High levels of NFH were noted in patients with SE and repetitive generalized tonic clonic seizures in comparison to control subjects ⁶². However, no study correlated NFH levels with the clinical outcome of patients.

Neurogenesis

Fluid biomarkers may reflect the SE severity as well as neuroprotective mechanisms resulting from the brain adaptation to excitotoxicity. Nevertheless, the current knowledge about neuroprotective mechanisms in humans after SE is scarce. Progranulin (PGRN) is responsible for neurite outgrowth and limits exceeding neuroinflammatory responses. Growing evidence suggests a role for PGRN in the lysosome.

In patients, increased levels of PGRN were measured in the CSF after SE and after a single tonic-clonic seizure, but no significant difference was observed between the two groups ²⁴. No correlation between progranulin levels and the time of SE was found. Additionally, higher CSF PGRN levels had no impact on clinical outcome. Nevertheless, the cohort was heterogeneous and not fully sufficient. A larger cohort with more homogenous patient is needed to further investigate the relationship between SE and PGRN.

The role of PGRN was investigated in rats after pilocarpine-induced SE ⁶³. An increase of mRNA and the protein is reported in the cortex and hippocampus. The increase of PGRN was delayed and peaked 48h-96h after the SE onset. PGRN elevation occurs primarily in activated microglia that could explain the elevation delay. Raising cerebral PGRN levels may contribute to neuroprotection by activating endolysosomal system and facilitating regeneration. PGRN could therefore be a potentially new therapeutic strategy in SE.

Neurotrophic factors, involved in the survival of neurons as well as in the proliferation and differentiation of neuronal precursors, could be useful as prognosis biomarkers in SE. An up-regulation of neurotrophic factors after SE is seen as reflecting a potential neuroprotective role⁶⁴. Moreover, it was shown that FGF-2/BDNF expressing vectors in pilocarpine model of SE were associated with an increased neurogenesis, limited neuronal damage and reduced occurrence of spontaneous seizures ⁶⁵.

Other potential biomarkers

This review reports a number of potential protein biomarkers in CSF and blood that were reported in SE. Since a few years, other types of fluid biomarkers are studied.

CSF microRNAs are promising biomarkers of temporal lobe epilepsy and SE; 20 microRNAs showed differential expression between patients in SE and controls patients ⁶⁶. Targets of these microRNAs include proteins regulating neuronal death, neuroinflammation, or gliosis; such mechanisms are involved in the late consequences of SE. Therefore, this new approach can be used to distinguish SE from other neurological diseases.

Lipids could provide a new source of biomarkers in SE as well. Indeed, disturbances in brain cholesterol homeostasis were reported in neurological disorders where excitotoxicity plays an important role ⁶⁷. Moreover, the accumulation of intracerebral cholesterol is associated with neuronal loss and epileptic abnormalities ⁵⁰. The availability of lipid biomarkers measurable at the level of the brain as well as in the serum or the CSF may represent new tools for prospective prognosis studies. Endogenous neurosteroids were studied in SE. They can modulate the excitability of neurons through effects on the GABA_A receptor chloride channel complex. Two recent studies reported low levels of neurosteroids (i.e. allopregnanolone and progesterone) in CSF of SE patients, suggesting their possible use as a novel therapeutic agent ^{68,69}. The first-in-man allopregnanolone use in adult super-refractory SE provided proof of principle to support its clinical development as a novel therapeutic agent ⁷⁰. Nevertheless, a phase 2 trial failed to demonstrate efficacy ⁷¹.

Some biomarkers are more specific to one clinical subtype of SE or aetiology. Therefore, it could be useful to determine specific biomarkers of new-onset refractory status epilepticus (NORSE), that is the most severe form of SE.

Conclusion

A large number of biomarkers related to SE reflecting injury of different cell types or structures within the CNS can be measured in CSF and blood. This review summarizes findings of animal and human studies, which were conducted to identify diagnosis and prognosis biomarkers of SE.

Diagnosis of convulsive SE relies mainly on clinical assessment. On the other hand, the diagnosis of non-convulsive SE relies on EEG recording. In this condition, non-EEG biomarkers, like neuronal or axonal injury proteins, could be of interest for the diagnosis and management of non-convulsive SE.

Biomarkers could also be useful to identify the aetiology of SE. Increased NSE levels is further specific of clinical subtypes than for SE aetiology. On the other hand, early increase of cytokines levels, such as IL-1 β , IL-6 or S100-B, should be more frequently associated to infectious or auto-immune SE. These biomarkers could guide the initiation of specific treatment.

Outcome scores based on demographic, clinical and EEG condition are already available. However, they mostly predict the mortality, while the severity of brain injury in survivors is poorly understood. Highest levels of NSE, tau protein and GFAP were correlated to the worst outcome. Elevated levels of serum NSE after generalized tonic-clonic seizures and SE could be primarily due to an increased diffusion of NSE across a more permeable blood-brain barrier, causing a transfer from the brain compartment to the serum compartment. Serum NSE might also be a relevant biomarker of seizures, independent of neuronal death. Otherwise, this explanation would not account for the increase of NSE in the CSF.

Further studies with longitudinal follow-up of these biomarkers and clinical data are needed to evaluate their prognosis value, in particular their ability to prospectively identify patients developing subsequent epilepsy or to prognose the cognitive outcomes.

To the best of our knowledge, no biomarker described in this review is disease-specific. They were also reported in mild traumatic brain injury (acidosis, NSE, S100-B, cytokines, albumin CSF/serum ratio), in stroke (NSE, S100-B, cytokines, progranulin), or in infection for cytokines^{8,72,73}. A good fluid biomarker is a molecule whose measurement in fluid provides highly sensitive and specific results. A compromise should be defined between the performance of specificity and sensitivity. This could be represented in receiving operator characteristics (ROC) curves to appraise the value of the biomarker and to identify the thresholds (Fig. 4, according to Freund et al.⁴⁷). No study of biomarkers in SE reported ROC curves in order to identify their performances. Moreover, among the reports of biomarkers of interest, only one was conducted in accordance with guidelines to uniformly report studies of body fluid biomarker in neurologic disorders⁷⁴. Nevertheless, this study aimed to establish the incremental prognosis value of S100-B after seizures, and so did not concern SE⁴⁷. According to the Simon's classification, other studies had also the lowest levels of evidence that might be used to determine the clinical utility of a biomarker due to an inappropriate design⁷⁵. We therefore cannot rank the different biomarkers in terms of performance. More studies following these guidelines are needed to validate their clinical potential. We also recommend also conducting separate studies with a specific design in order to evaluate respectively biomarkers for SE diagnosis, for specific aetiology or for prognosis.

A good fluid biomarker is a molecule whose biochemical assessment is easy, quick and reliable and which is stable in the body fluids. We can appraise these different biomarkers on the practicality of measuring. Firstly, we can range biomarkers described above in three categories according to the mean duration of biochemical assessment: hours for acidosis biomarkers, S100-B and NSE; days for albumin ratio and cytokines levels and up to several weeks for progranulin and tau proteins. Secondly, the reliability of the biochemical

assessment should be taken into account. Data may be more reliable with an automatic method, used in the measurement of S100-B and NSE levels, compared with manual methods, used for the evaluation of progranulin or tau levels. Nevertheless, NSE is highly sensitive to haemolysis and an adapted pre-analytic approach is needed for a good monitoring. Thirdly, some biomarkers are less stable in body fluids. For example, IL-1 β is a less stable molecule when compared with IL-6 and S100-B protein has a shorter half-life compared with NSE, explaining why time that elapses between the seizure occurrence and the samples could induce variable results. Based on their physical and biochemical characteristics, NSE seems to be the best valuable biomarker.

Identification of suitable biomarkers could help clinicians establish a more accurate diagnosis, in particular for the non-convulsive type of SE, to identify SE aetiology, to predict clinical outcome and to manage the treatments. Biomarkers would be combined with clinical and paraclinical data (EEG and MRI) for an overall view of a patient admitted in an intensive care unit with SE.

Key Points

- Refractory status epilepticus is associated with molecular and cellular changes that may induce brain injury with neuronal loss and subsequent epilepsy
- Specific biomarkers can be detected in the fluids and the brain tissues obtained from patients and animal models of status epilepticus
- Neuron Specific Enolase protein is a useful biomarker of the neuronal loss for which highest levels were correlated with the worst outcome
- Elevated cytokines levels could suggest infectious or auto-immune status epilepticus and identify specific etiologies requiring individualized treatments

- Further studies with longitudinal follow-up of these biomarkers and clinical data are needed to better evaluate their prognosis value in status epilepticus

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V.N. reports personal fees from UCB, Liva Nova, and Eisai, outside the submitted work. Others authors report no disclosures.

Ethical Publication Statement

We confirm that we have read the Journal’s position on issues involved in ethical publication and affirm that this report is consistent with those guidelines

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Figures legends

Fig. 1: Progressive brain MRI changes in a 17-year-old patient suffering from new-onset prolonged refractory status epilepticus.

MRI was performed a few days after the onset of a generalized status epilepticus (A, C) and after 8 weeks (B, D). Axial FLAIR sequences (A, B) showed an initial hypersignal in the claustrum and the pulvinar (arrows). Coronal T1 sequences (C, D) showed a major atrophy on the second MRI (D), affecting both cortical and sub-cortical regions, i.e enlargement of ventricles and hippocampal atrophy (arrows).

Fig. 2: Major EEG changes during status epilepticus

Extract of an EEG (A), of a bipolar montage, from the same patient, as in Fig.1, showing long periods of isoelectric activity (first seconds of the trace) related to high doses of barbiturate, that alternate with repetitive seizures starting and ending with high-amplitude spikes. Time frequency analysis (B) of the EEG signal shows the spectral signature of a seizure.

Extract of an EEG (C), of a bipolar montage, from a 20-year-old patient presenting with a refractory status epilepticus related to a NMDAr encephalitis. The EEG shows a continuous waxing and waning of ictal activity.

Time frequency analysis (D) of a longer period (10 minutes) showing the spectral signature of two seizures. The spectral signature of the seizure C is shown in the dotted box.

Fig. 3: Biomarkers related to the pathophysiology of status epilepticus.

During status epilepticus molecular and cellular changes occur: neuronal death is followed by the release of Neuron Specific Enolase (1) and neuroinflammation with production of cytokines (2). The blood brain barrier is disrupted; albumin may worsen the inflammatory process (3) and leukocytes can be attracted (4). All these processes result in gliosis (5) that

can be identified by GFAP and S100-B analysis. All these molecules could cross the blood brain barrier and be measured in the blood samples (6).

Abbreviations: BBB, blood brain barrier; GFAP, Glial Fibrillary Acidic Protein; IL, interleukin; NSE, Neuron Specific Enolase.

Fig. 4: Example of a receiving operator characteristic curve for S100-B.

Reprinted from *Predictive Value of S100-B and Copeptin for Outcomes following Seizure: the BISTRO International Cohort Study* (p.8), Yonathan Freund, 2015, PLOS One.

This study evaluated the performance of S100-B in addition to clinical variables in predicting outcome of patients following seizure. A receiving operator characteristic curve was constructed. It could be used to appraise the value of a biomarker by calculating the area under the curve and to identify thresholds using the Youden's method. A sensitivity of 0.57 and a specificity of 0.53 for S100-B were defined.

The best biomarker would have simultaneously a sensitivity around 100% and a score "1-specificity" around zero.

Pathophysiology	Biomarker	Research findings in CSF / brain tissues		Research findings in blood		Prognostic value	Advantages	Current limitations
		Animal models	Humans	Animal models	Humans			
Acidosis	Lactate	/	Levels are elevated in patients with generalized SE ²⁸	/	/	Magnitude of elevation as indicator of morbidity or mortality ²⁸	Quick biochemical assessment	Not specifically associated to SE No study performed on non-convulsive SE
	pH	/	/	pH fell five minutes after the induction of epileptic seizures ³⁰	/	/		
Acute neuronal injury	NSE	/	Increase of NSE levels in patients with cryptogenic/symptomatic SE ³⁵	Elevated levels in a lithium pilocarpine animal model of SE ²¹	Levels peak at 24-48 hours after the onset of SE, normalized at 7 days ³⁶ Highest level in subclinical generalized convulsive SE ³⁷	Outcome highly correlated with the peak of serum NSE ^{36,37}	Good neuronal specificity High stability in body fluid Correlation to neuronal death, duration and outcome of SE	Highly sensitivity to haemolysis Serum levels could be decreased by two major antiepileptic drugs (bias for prognostic evaluation)
Blood-brain barrier breakdown	CSF-serum albumin ratio	/	Increase of the ratio in patients with SE and generalized tonic-clonic seizures in comparison to controls ^{35,42}	/	/	/	/	Not specific to SE Lumbar puncture not systematically performed in clinical assessment
Gliosis	S100-B	Increase from the first day to the late period (56 days) ⁴⁸	/	Decrease of S100-B at one day after the lithium-pilocarpine injection and elevation at 14 days ⁴⁸	Higher S100-B levels in epileptic patients ⁴⁷	Higher S100-B serum levels associated with unfavorable outcome ⁴⁷	Almost brain specific Release in blood only in case of BBB breakdown	No study conducted in blood or CSF of SE patients

	GFAP	Increase staining in hippocampus ⁵⁰	Elevated CSF GFAP levels in children after seizures ⁴⁹	/	/	CSF GFAP correlated with SE severity ⁴⁹	No extracerebral production of GFAP	Need more study with larger cohorts and longitudinal follow up to validate its prognostic interest
Neuroinflammation	IL-1β	Increase of IL-1 β mRNA in the hippocampus ^{56,57}	Increase of IL-1ra and decrease of IL-1 β ⁵²	/	Discordant results ^{53,54}	/	/	Unstable molecule
	IL-6	/	/	Increase blood levels in all studies ^{53,58}	Increase blood levels in all studies ^{53,58}	/	/	Unstable molecule
	HMGB1	Increase staining after kainic acid and in human epileptic tissues ⁵¹	/	/	/	/	Good stability in blood	No study conducted in human body fluids
Axonal injury	Tau	/	Higher CSF levels in 50% of patients with SE ⁶⁰	/	/	Higher CSF tau levels in patients with refractory SE, disabilities and chronic epilepsy ⁶⁰	Correlation to SE outcome	Influence of anesthetics drugs No study conducted in blood samples
	NFH	/	Higher levels in patients with SE and repetitive generalized tonic clonic seizures ⁶²	/	/	/	/	No study conducted in blood samples
Neurogenesis	Progranulin	Increase of the mRNA in the hippocampus and cortex at 48-96 hours ⁶³	Increased levels of PGRN in patients with SE ²⁴	/	/	No impact on clinical outcome ²⁴	/	No study conducted in blood samples

Table 1: Potential CSF and blood biomarkers in status epilepticus.

Summary of clinical and preclinical studies.

Abbreviations: NSE, Neuron Specific Enolase; GFAP, Glial Fibrillary Acidic Protein; HMGB1, High Mobility Group Box 1, NFH, Neurofilament Heavy Chain Protein