REVIEW ARTICLE

Molecular and cellular biomarkers in status epilepticus and epilepsy

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Short title: *Molecular and cellular biomarkers in SE and epilepsy*

What is not yet known on the issue addressed in the submitted manuscript

Molecular and cellular biomarkers in combination with imaging and electrical investigations will provide a more specific platform for defining epileptogenic zone.

Research hypothesis

Analysis and selection of the most informative molecular and cellular biomarkers predictive of epileptogenesis.

The novelty added by manuscript to the already published scientific literature

Peripheral biomarkers have numerous uses in the treatment, prognosis, and pharmacovigilance of epilepsy. Epilepsy is represented by a research area of specific biomarkers, which are of great clinical importance, being necessary for forecasting the disease, the risk of developing neurological sequelae and pharmacoresistant to antiepileptic remedies. Thus, their identification could have a significant impact on the clinical course of the disease.

Summary

Introduction. Peripheral biomarkers have numerous uses in the treatment, prognosis, and pharmacovigilance of epilepsy. Unfortunately, no peripheral biomarker has demonstrated proven efficacy, although several options are being investigated. In this article, we want to analyze the main areas in which peripheral biomarkers can present their usefulness, including participation in the processes of inflammation, dysfunction of the blood-brain barrier, changes in metabolism, hormones, and growth factors.

Material and methods. Publications on diagnostic biomarkers of epilepsy were reviewed. References were identified by PubMed, MEDLINE and Scopus search until June 2022, with various combinations of the terms – „epilepsy", „seizures", „epileptogenesis", „biomarkers", „neuroimaging", „inflammation", „status epilepticus", „prognosis". A qualitative and analytical study was performed focused on primary studies published in 2020-2022. More than 85 sources were identified and 33 were selected for analysis from the PubMed, MEDLINE, and Scopus online databases. 12 articles, 5 clinical trials, 2 meta-analyses, 7 reviews, and 7 systematic reviews were identified.

Results. Screening articles from online databases according to the search criteria, we found 258 titles on molecular and cellular biomarkers in epilepsy highlighted. The final bibliography included 33 sources that summarized that biomarkers of epileptogenesis are expensive and difficult to research, but the identification of biomarkers specific to the entire epileptogenic process, in close proximity to neuronal damage, have demonstrated the possibility of predicting the risk of seizures, epilepsy and resistance to treatment.

Conclusions. Epilepsy remains a continuous area of research; a special role is occupied by specific biomarkers of great clinical importance, being necessary for the prognosis of the disease, the risk of neurological sequelae, refractory to anti-epileptic drugs. Thus, their identification could have a significant impact on the clinical course of the disease.

Keywords: biomarkers, epilepsy.

Introduction

Epilepsy affects approximately 50 million people worldwide. One-third of them show drug resistance to antiepileptic drugs [1-3]. Specific biomarkers essential to determine drug resistance are not currently established. The biomarkers are defined as „cellular, biochemical or molecular changes, which can be measured in biological environments, such

as human tissues, cells or fluids" [4, 5]. More recently, a working group of the National Institutes of Health (NIH) extended this definition to „a characteristic that can be objectively measured and that is an indicator of normal and pathological biological processes or pharmacological response to applied therapeutic intervention" [6]. In the case of epilepsy, the spectrum of biomarkers ranges from neuroimaging and electrophysiological markers to molecular and cellular markers, determined in peripheral fluids and tissues.

This article is a review article on soluble biomarkers, determined in blood and cerebrospinal fluid (CSF). They may have multiple potential uses such as the ability to predict the development of epilepsy following a brain injury and/or after a first seizure, the progression of the disease and its severity, and the possibility of developing drug resistance to antiepileptic drugs [4]. Thus, bibliographic sources were analyzed in which data related to the importance of biomarkers in epilepsy were exposed.

It is known that the biomarkers of epileptogenesis are expensive and difficult to research. Even after severe brain conditions, in the presence of a potential epileptogenic risk, such as severe head trauma, the proportion of people who develop epilepsy is not high. Moreover, this process can take decades. An ideal situation would be to identify the biomarkers specific to the entire epileptogenic process, in close proximity to neuronal injury, with the possibility of predicting the risk of developing seizures, epilepsy, and resistance to treatment [7, 8].

Molecular and cellular biomarkers should ideally be present in an accessible compartment such as blood, tissues, CSF, sputum, or urine (Figure 1) [9].

Material and methods

Publications on diagnostic biomarkers of epilepsy were reviewed. References were identified by a search in the PubMed, MEDLINE and Scopus databases for articles published until June 2022, with various combinations of the terms – „epilepsy", „seizures", „epileptogenesis", „biomarkers", „neuroimaging", „inflammation", „status epilepticus", „prognosis". A qualitative and analytical study was performed focused on primary studies published between 2020-2022 and dedicated to the identification of biomarkers for diagnosis, disease progression, and complications of epilepsy. The articles contain a detailed analysis and a synthesis of recommendations regarding the targeted selection of biomarkers in the diagnosis of epilepsy. Articles were selected taking into consideration the title and their abstracts. More than 85 sources were identified and 33 were selected for analysis. 12 articles, 5 clinical trials, 2 meta-analyses, 7 reviews, and 7 systematic reviews were identified.

Results and discussion

Inflammation

Recent experimental studies have revealed that inflammation can precipitate seizures and support convulsive activity [10-12]. In addition, it can influence epileptic discharge by changes in glutamate and ions homeostasis involved in the epileptogenic process. Consequently, biological markers of inflammation represent a pathway for identifying patients in whom inflammation plays a key role in epileptogenesis and/or maintaining neuronal excitability [10, 11]. Furthermore, immunomodulatory drugs, including steroids and intravenous immunoglobulins, have been shown to have successful therapeutic effects in some children with epileptic encephalopathies, which are often resistant to conventional antiepileptic drugs (AED).
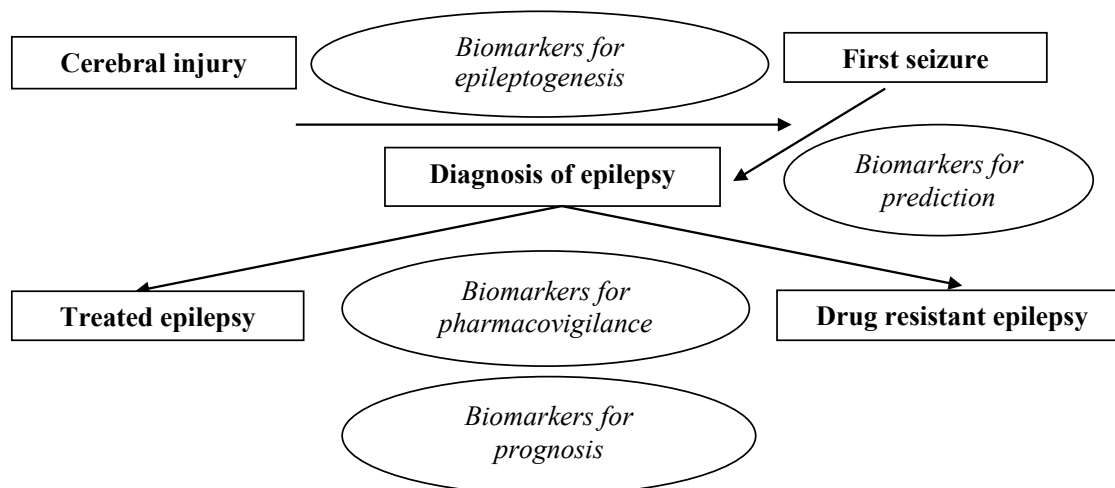
Fig. 1. Types of biomarkers involved in pathogenetic process of epilepsy.

Inflammation of the tissues can be involved not only in the generation of seizures but also in the development of the drug resistance phenotype. Surprisingly, even children

with focal seizures, seizures that are not traditionally considered to be inflammatory in nature, have a positive response to steroids. Targeting the inflammatory process may

present a novel therapeutic strategy in the treatment of epilepsy and circulating biomarkers capable of demonstrating the response to treatment have an increased value [12, 13].

In several studies, individuals with drug-resistant focal epilepsy have been shown to exhibit an imbalance in the ratio of interleukin-1 / interleukin-1 receptor antagonists (IL-1/IL-1RA). IL-1 is recognized as a mediator of brain inflammation. In rodents, pharmacological blocking of IL-1 biosynthesis significantly reduces the risk of developing seizures. This "pro-inflammatory cytokine profile" in peripheral blood, consisting of elevated IL-6 levels with a low IL-1/IL-1RA ratio, may be found among patients in whom persistent and unsolved inflammation leads to the development of neuromodulation associated with changes in neuronal excitability. Likewise, a higher concentration of IL1-Elm in serum and CSF was associated with an increased risk of developing epilepsy after brain damage [14].

The high-mobility group box-1 (HMGB1) is one of the best-known mediators of neuroinflammation evoked by epileptogenic lesions, a fact proven in clinical models of epilepsy [15]. It is actively released by immune cells during injury-induced infection or aseptic inflammation. Cellular death leads to the passive release of HMGB1. Experimental models of epilepsy suggest that the acetylated disulphide form of HMGB1 is responsible for the occurrence of inflammation in epilepsy. A pilot study in patients with resistant focal epilepsy, suggests that HMGB1 isoforms can be considered candidate biomarkers in epilepsy stratification [16]. However, HMGB1 is not only specific for epilepsy and in fact, it is a sensitive and specific biomarker for several conditions, including autoimmune pathologies and malignancies.

Pharmacological inhibition of HMGB1 has been successful in numerous experimental disease models [17]. Interventions used included direct inhibition using polyclonal and monoclonal antibodies, competitive HMGB1 a-Box inhibitors, HMGB1 sequestration, and degradation methods. Since HMGB1 has no cerebral specificity, it is unclear whether peripheral or central nervous system (CNS) changes are responsible for seizures, or the therapeutic effects described above.

Blood-brain barrier

Dysfunction of the blood-brain barrier following prolonged seizures in animal models has been recognized since the 1950s. Vasogenic edema, caused by neurovascular changes, was first described by Klatzo and colleagues. In some cases, dysfunction of the blood-brain barrier can cause seizures, while dysfunction created artificially by other means leads to delayed epileptogenesis [18, 19]. Impaired blood-brain barrier function due to a hypertensive crisis in eclampsia and hypertensive encephalopathy may involve changes in serum magnesium levels. Injury to the blood-brain barrier in experimental studies caused the delayed occurrence of seizures [20, 21]. Impaired blood-brain barrier function is commonly found in various neurological conditions, such as encephalitis, meningitis, stroke, Alzheimer's disease, and other CNS disorders. There is no doubt that cerebrovascular dysfunction conditions or sustains

seizures. The role of cerebrovascular impairment in various CNS disorders, including epilepsy, has been clinically accepted in the past, but only recently, it has been tested as an important mechanism underlying epileptogenesis. In the case of human epilepsy, the results of the studies are suggestive for a loss of selective permeability of the blood-brain barrier in the focal regions from which the convulsions originate [22, 23].

In addition, data from several studies support the idea that the blood-brain barrier in patients with epilepsy exhibits a variety of molecular changes that are in one way or another involved in epileptic disease [18-20]. Given the importance of the blood-brain barrier for convulsive disorders and epilepsy, it is not surprising that biomarkers in this pathophysiological field are highly examined, but at the same time, they require further research. In general, there are three approaches to determine the integrity of the blood-brain barrier in epilepsy. They do not differ from aspects previously used to measure cerebrovascular integrity in other neurological diseases [7]. Historically, the ratio of serum albumin to CSF albumin was the first used approach. It is known that the vascular barrier protects the brain from harmful substances in the blood while providing the necessary nutrients to the brain for proper functioning and strict regulation of the traffic of cells and molecules from the blood to the brain. The vascular barrier also separates impermeable macromolecules (>~500 Da) in the blood and brain. Thus, when the blood-brain barrier is intact, the albumin is 10 times more concentrated in the blood and will have a constant ratio to the concentration in the CSF [20]. A similar principle, but applied in another way, would be the nuclear magnetic resonance imaging (MRI) with the application of contrast agents. In this case, the „ratio" of the cerebral parenchyma to the blood is measured topographically, and the distribution of the substance injected into the blood is visualized in the brain. The absence of extravasation indicates an intact blood-brain barrier. Thus, the brain produces specific proteins that are isolated in the CNS in conditions of the intact blood-brain barrier. In there is a lesion of the blood-brain barrier, proteins, which are normally present in high concentrations in the CNS, are free to diffuse into the blood according to their concentration gradient. An ideal peripheral biomarker of clinical significance, should be: (1) a protein (or molecule) present in small or undetectable concentrations in the serum of healthy subjects; (2) present in the brain and CSF, being in a higher concentration in the cerebral parenchyma than in plasma; (3) available for extravasation in case of damage to the blood-brain barrier; and, (4) further released by brain cells in response to brain damage (for example, during reactive gliosis). Several proteins, including S100 calcium-binding protein B (S100B), neuronal-specific enolase (NSE), and glial fibrillary protein (GFAP) have been evaluated for their functions, and the S100B protein meets all the above-mentioned criteria. The imaging techniques available for clinical research do not have the high resolution required to „visualize" this structure, although the contrast agents that have been used to

measure the integrity of the blood-brain barrier. Functional assessment of the status of the blood-brain barrier by calculating the albumin coefficient (QA) in serum and CSF and MRI with contrast is widely accepted as the gold standard for determining the integrity of the blood-brain barrier. Recent work has shown that the serum level of the S100B protein correlates with QA, thus allowing the measurement of the CSF protein indirectly and without performing a lumbar puncture [21, 22].

It is relevant that serum S100B protein is the most studied marker, which may be involved in the mechanisms of delayed epileptogenesis after traumatic brain injury. Brain injury, in general, is associated with a rapid loss of integrity of the blood-brain barrier followed by the development of brain disease. Thus, the protein S100B emerged as a peripheral biomarker indicative of the permeability of the blood-brain barrier. The increase in serum levels of S100B reflects the presence of a damaged barrier and can predict long-term consequences after a brain pathology. Intracranial events are associated with an increased risk of seizures; thus, it is possible that the S100B protein will also prove to have a utility in detecting people at increased risk for seizures. Two independent meta-analyses for S100B in traumatic brain injury concluded that normal S100B levels accurately predict the results of a normal neuroimaging exam and that S100B sampling within 3 hours of injury should be considered when there is no focal neurological deficit or significant extracerebral injury [24, 25]. Therefore, there is an opportunity to conduct a test with a high predictive level, so that many unnecessary scans can be avoided, a test with a predictive value to diagnose complications after brain trauma.

CCL-2 is a pro-inflammatory chemokine produced by hyperactive neurons and microglia, it is involved in immune cell migration, chemoattraction of monocytic cells, disruption of blood-brain barrier and neuroinflammation. Its production is stimulated by IL-1β and tumor necrosis factor α (TNF-α) in the microglia, vascular endothelial cells, pericytes and neurons. The activation of CCL2 initiates the adhesion of monocytes to the inflamed endothelium, resulting in infiltration into the brain parenchyma [26, 27].

Aquaporin 4 (AQP4) is a membrane protein, serving as a water channel in conformity to the osmotic gradient; it is expressed by glial cells in the brain and the spinal cord. These channels are localized in the astrocytic plasma membrane that abut on brain micro vessels or on the pial covering of the brain surface. Both lowered expression and incorrect localization of AQP4 on end-foot membranes can lead to an alteration in the astrocytic function and homeostasis. The astrocytic AQP4 channels mediate water clearance in vasogenic edema with exacerbation of intracellular edema and so lead to cellular edema in the CNS. It was found that abnormalities in astrocytic ion channel expression, localization, and function could cause alteration of ion homeostasis and neurotransmitters, deposition of characteristic proteins, oxidative stress, and neuroinflammation. The overexpression and altered localization of AQP4 channels in astro-

cytes has been suggested as a reason for blood–brain barrier (BBB) dysfunction, manifested by the reduction in the flow of brain interstitial fluid mediated by AQP4 and due to a failure of clearance accumulation of pathologic markers such as amyloid-β (Aβ), and mutant α-synuclein (α-SYN) which trigger the production of reactive oxygen species (ROS) and inflammatory factors and contributes to the pathological progression of neurodegenerative diseases [28, 29].

Scientific evidence maintains the hypothesis that brain tissue inflammation is a central component for the development of seizures. Toll-like receptors (TLR) 4 is a trans-membrane protein that is highly expressed in the neurons, astrocytes, and microglia under pathological situations, such as cell injury and epileptic seizure [30, 31]. A study suggests a significant association between homozygous CC and heterozygous CT genotypes of TLR4 and epilepsy. It identifies these genotypes as a risk factor for epilepsy [32]. Some studies in rat models suggest that TLR4 signaling takes part in the drug-resistant epilepsy pathway. Inhibition of TLR4 downregulates IL-1β, TNF-α and NF-κB at the cellular level. The same study reveals that inflammatory pathways in drug-resistant epilepsy are activated. Such inflammatory factors as TLR4, IL-1β, TNF-α, NF-κB signaling factors and P-glycoprotein seem to be involved in the genesis of a drug-resistant epilepsy network. TLR4 was proposed to be an upstream regulator, that activates the downstream NF-κB, regulates inflammatory factors IL-1β, TNF-α, and other cytokines [33].

Conclusions

Epilepsy is represented by a research area of specific biomarkers, which are of great clinical importance, being necessary for forecasting the disease, the risk of developing neurological sequelae, and refractory to antiepileptic remedies. Thus, their identification could have a significant impact on the clinical course of the disease. It should be recognized that many of the biomarkers discussed in this review are also involved in other pathologies, including non-neurological diseases. Prospective research will be needed to identify individual or groups of specific biomarkers that would differentiate epilepsies from other diseases. New therapeutic strategies should involve integrating clinical information, including electroencephalography and neuroimaging exams, with new molecular and cellular biomarkers.

Identifying biomarkers of aberrant inflammation could stratify patients to determine who contributes to maintaining epileptic status. Focusing on inflammation, which plays a critical role in epilepsy may encourage the development of various strategies that halt the progression of the drug-resistant phenotype.

Markers of the integrity of the blood-brain barrier are useful tools for determining sequels in a variety of neurological diseases or acute events (strokes, trauma). Currently, the role of these markers in the prognosis and diagnosis of convulsive disorders is being studied, although, from, another point of view, these markers have already demonstrated that the blood-brain barrier is damaged now of

convulsive seizures and that the disruption of the integrity of this barrier is epileptogenic. Moreover, when the blood-brain barrier is damaged, allowing albumin to enter the interstitial space, can affect the effectiveness of some drugs and, therefore, markers of barrier integrity can be useful in therapeutic decision-making.

Better tools to predict the onset of epilepsy could lead to the development of new therapeutic strategies to prevent the development of epilepsy, potentially in the form of immunomodulatory intervention. In addition, early prediction of drug resistance would mean that patients could be evaluated for epilepsy surgery at an early stage, thus avoiding the administration of more antiepileptic drugs that are associated with side effects, are inevitably doomed to fail.

Declaration of conflict of interests

The authors have no conflict of interests to declare.

Authors' contribution

CC, IC, AC, LF, OC – the conception and the design of the study, the acquisition of data, the drafting of the manuscript; SH, SG – the critical revising of the manuscript for important intellectual content and the approval of the version of the manuscript to be published. All the authors – the analysis and the interpretation of data.

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