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Role of microRNAs As Biomarkers in Sepsis-Associated Encephalopathy

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Abstract

Sepsis-associated encephalopathy (SAE) is a neurological complication of sepsis, characterized by brain dysfunction without any direct central nervous system infection. The diagnosis of SAE is currently a challenge. In fact, problems in making a diagnosis of SAE cause a great variability of incidence that can reach up to 70% of all septic patients. Even more, despite SAE is the most frequent type of encephalopathy occurring in critically ill patients, the molecular mechanisms that guide its progression have not been completely elucidated. On the other hand, miRNAs have proven to be excellent biomarkers for both diagnosis and prognosis, especially in brain pathologies because of their small size they can cross the blood–brain barrier easier than other biomolecules. The identification of new miRNAs as biomarkers may help to improve SAE diagnosis and prognosis and also to design new therapies for this clinical manifestation that produces diffuse cerebral dysfunction. This review is focused on SAE physiopathology and the need to have clear criteria for its diagnosis; thus, this work postulates some miRNA candidates to be used for SAE biomarkers because of their role in both, neurological damage and sepsis.

Keywords Sepsis-associated encephalopathy · Sepsis · Biomarkers · microRNAs

Introduction

According to the NIH, encephalopathy is defined as any diffuse disease of the brain that alters brain function or structure [1]. Sepsis-associated encephalopathy (SAE) is a brain dysfunction due to sepsis, and it is linked to a systemic inflammatory response syndrome (SIRS) [2, 3]. In sepsis, a potentially fatal organ dysfunction through a dysregulated host response to infection occurs (Sepsis-3 guidelines 2019) [4]. This process can progress to septic shock, a subtype of sepsis in which circulatory, cellular, and metabolic abnormalities are associated with a dramatic increase in mortality [4]. Therefore, SAE is a multifactorial syndrome categorized as a diffuse brain dysfunction induced by the systemic response to an infection, in the absence of direct central nervous system (CNS) infection or other types of encephalopathy, and it can appear as the first organic dysfunction in many septic patients [5, 6]. Sepsis has a global incidence of around 48 million cases per year and causes 11 million deaths, being the leading cause of death around the world, even more than acute myocardial infarction [7–9]. SAE incidence is predicted between 9 and 71% of septic patients, depending on the inclusion and exclusion criteria used [3, 10–13]. In fact, SAE is the most common cause of encephalopathy in the intensive care unit (ICU) worldwide [14–16], but the disparity of data and incidence of SAE at ICUs suggests that new and more specific criteria for SAE diagnosis are needed.

Despite sepsis is associated with high mortality incidence, about two out of every three patients survive a sepsis episode. In addition, sepsis can compromise the quality of life in survivors as they have sequelae after sepsis, including

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immunosuppression, increased cognitive impairment, depression, increased cardiovascular risk, and post-traumatic stress disorder [17–19]. Furthermore, sepsis is associated with increased morbidity and mortality, as occurs in SAE, which is characterized by psychiatric disorders and long-term cognitive impairments in sepsis survivors [6, 20–24]. Moreover, despite SAE is described as an acute reversible syndrome in most cases, there is mounting evidence that there are many substantial risks for long-term cognitive impairments. It appears to be linked to direct cellular damage in the brain, mitochondrial and endothelial dysfunction, neurotransmission disturbances, and imbalances of calcium homeostasis in brain tissue, which may impair learning memory and cognitive function [25, 26]. Disturbances in mental processing speed, executive function, attention, and visual-spatial disabilities are other features associated with SAE [5]. Furthermore, it is noteworthy that patients with lower scores on the Glasgow Coma Scale (GCS) and higher Acute Physiology and Chronic Health Evaluation II (APACHE II) scores are more likely to suffer SAE [13].

Diagnosis of SAE represents a challenge due to lack of specific test or diagnostic criteria to define the SAE condition, as well as the scarce number of biomarkers available or these available biomarkers have low specificity and sensitivity [27–30]. However, although currently available tests do not allow a specific diagnosis of SAE, these tests actually help to exclude other pathologies [5, 12, 31]. In fact, nowadays, SAE is being diagnosed by ruling out direct infection or primary pathologies in the CNS, or consequence of deleterious effects produced by different drugs, toxins, and metabolic disorders [6, 12, 31]. A study performed by Zhang et al. [13] showed that patients admitted to ICU usually manifest pre-existing or chronic kidney or liver failure, blood glucose disturbances, electrolyte imbalances, or pre-existing CNS disease, among others. For all these reasons, SAE is managed as sepsis; so the approach is based on controlling properly metabolic disturbances and avoiding neurotoxic drugs [12, 27, 31].

Clinically, SAE is manifested in two different forms: an early predictable form and a late form, which is usually accompanied by complex metabolic encephalopathy that can lead to irreversible brain damage [11]. It is noteworthy that sometimes SAE is associated with high levels of aromatic amino acids (AAA) in plasma [32] as a consequence of energy deficit, which in turn causes a breakdown of the metabolic pathways related to gluconeogenesis and obtain energy in muscle [33, 34]. The muscles are able to degrade branched-chain amino acids but not AAA, causing their increase in plasma [33–36]. Higher levels of AAA have been observed in septic patients in comparison to healthy subjects [33, 34] and also in patients with SAE with no serious liver abnormalities [32]. Nevertheless, SAE can be induced by a hepatic failure, which is commonly present in septic patients and in patients suffering from chronic liver disease or cirrhosis [37–39]. Hepatic failure is highly related to encephalopathy (hepatic encephalopathy), where a high amount of amino acids released into the bloodstream are found, due to the impossibility of the liver to catabolize amino acids, especially the AAA [35, 40]. In addition, Basler et al. [32] demonstrated that as the disease progressed, the levels of the amino acid were unbalanced (the ratio of branched-chain to aromatic amino acids were decreased), while inflammation markers (such as procalcitonin (PTC) or IL-6) increased, sharpening as time passed. Furthermore, it established a relation between sepsis severity and the level of amino acids in plasma: those patients who did not survive sepsis had higher levels of aromatic and sulfur-containing amino acids (Met and Cys) in comparison to septic survivors [33, 34, 41]. Importantly, it was proposed that the outcome of septic patients might be positively affected using combined therapy with glucose, insulin, and branched-chain amino acids [33, 34, 42]. However, these results need further research.

SAE is characterized by decreased fluctuating attention and confusion in early stages that can progress to delirium, agitation, and coma in late stages [5, 12, 27, 31]. Notably, up to 70% of patients with advanced SAE show critical illness neuromyopathy [15].

It is noteworthy that according to the Sepsis-3 consensus, severe COVID-19 is related to sepsis [43]. Interestingly, COVID-19-associated acute brain dysfunction has been recently described including encephalitis, Guillain-Barré syndrome, ischemic and hemorrhagic stroke, and COVID-19 SAE [44]. These heterogeneous events occurring in some cases of severe COVID-19 patients may further compromise the clinical course and outcomes of severe COVID-19 patients [45]. Therefore, the identification of such events is very important for early empirical combination therapy to survive severe COVID-19 [44].

Physiopathology of SAE

Several mechanisms are involved in SAE pathogenesis, such as disturbance of the blood–brain barrier (BBB), neuronal apoptosis, endothelial activation, hyperinflammation produced by inflammatory cytokines release, oxidative stress, neurotransmission disturbances by alteration in neurotransmitter level, altered brain signaling, altered microcirculation, and dysregulated metabolism [5, 46]. Recently, Kodali et al. demonstrated that cerebral endothelial cells (CECs) are the first activated cells during the earliest stages of acute neuroinflammation, defined as a spinal cord or brain inflammatory response, mediated by cytokine, chemokine, and oxidative stress production [47, 48]. Kodali et al. [47] suggested that CECs are the main source of proinflammatory mediators, which in turn can

promote glial cell activation, such as microglial activation. Furthermore, Kodali et al. [47] observed that SAE continued by activating apoptotic signaling in CECs, which is known that causes a BBB disruption, allowing the entrance of peripheral cytokines into the CNS and thus causing an exacerbate gliosis. Finally, it causes a vicious neuroinflammatory cascade, which is commonly observed during SAE. This process is summarized in Fig. 1.

SAE patients present a hyperinflammatory response mainly in the hippocampus, which can be quantified by measuring the levels of the NLRP3 inflammasome, IL-1β, IL-6, and gliosis [2, 27, 31, 49]. The tumor necrosis factor alpha (TNF-α) is another cytokine released in the brain when SAE occurs [27, 31, 50]. Furthermore, the hyperinflammatory response was associated with the activation of the inflammasome in the microglia, being, the pyroptosis, an important nonapoptotic inflammatory cell death determinant of neurodegeneration [51]. In fact, Sui et al. [2] showed that the antioxidant resveratrol was able to inhibit the NLRP3 expression and IL-1β cleavage in a dose-dependent manner. They conclude that resveratrol treatment improved spatial memory and also decreased inflammation by inhibiting the NLRP3/IL-1β axis in the microglia in a mice model of SAE [2].

Moreover, it is now accepted that pyroptosis contributes to the development of many neurological diseases, including SAE [51, 52]. It is noteworthy that the CNS is able to recognize damage-associated molecular patterns (DAMPs) through pattern recognition receptors (PRR), which are present mainly on microglia and astrocytes. These receptors are localized on the membrane's surface, for extracellular signal recognition, and in the cell cytoplasm for intracellular signal transmission [52]. There exist many studies that demonstrated the expression of NLRP1, NLRP2, and NLRP3 in some CNS-related diseases, especially under stress conditions [53–57]. Neurons, astrocytes, and microglia are the main cells able to suffer pyroptosis in the CNS and thus able to express the pyroptosis-related cytokines (IL-1α, IL-1β, and IL-18) and the receptors related to this process [58–60].

It has been demonstrated that inflammation is able to increase cytokine transcription of IL-1β TNF-α and IL-6. This is of special relevance because IL-1β can activate microglia, which has neurotoxic properties when it produces the release of nitric oxide (NO) and reactive oxygen species (ROS) [5, 27, 31, 61]. TNF-α causes neutrophil infiltration in brain tissue, neuronal apoptosis, and brain edema [31]. Furthermore, at the early stages of neuroinflammation, it is able to cause neurotoxicity [62]. Meanwhile, IL-6 can

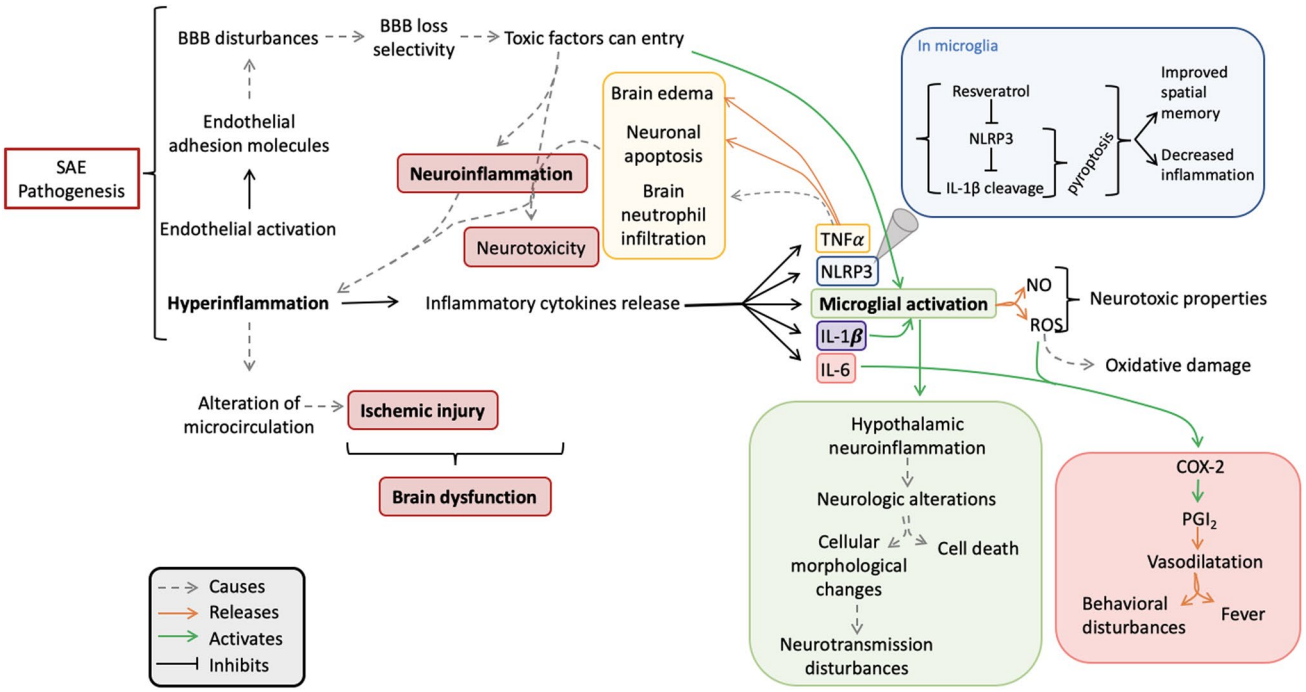

Fig. 1 Schematic overview of defined SAE physiopathology. This figure shows a normal physiopathology in a healthy brain and makes a comparison with an altered brain physiopathology, which is observed during SAE. An activation of CECs and the apoptosis induced on them causes a BBB disruption, which loses its selectivity, and an entrance of proinflammatory cytokines is produced. These processes promote glial cell activation, producing reactive astrocytes and activated microglia, which finally causes gliosis. Furthermore, in an SAE brain, an overexpression of glutamate as a consequence of neuroinflammation is produced. Ischemic lesions cause an altered microcirculation in the SAE brain. Finally, a vicious neuroinflammatory cascade produced during SAE, causing brain atrophy. BBB, blood–brain barrier; NT, neurotransmission; ROS, reactive oxygen species

induce cyclooxygenase-2 (COX-2) expression, which in turn increases the levels of the vasodilatory prostaglandin I2 (PGI_2) [31, 63], causing fever and behavioral disturbances [5]. Endothelial adhesion molecules, such as V-CAM and I-CAM, also increase their expression in cerebrovascular endothelial cells, increasing the permeability of the BBB [5, 31, 64] and allowing the transfer of toxic factors from the peripheral circulation to the brain [12, 27], causing the BBB loss of selectivity, leading to neuroinflammation and microglia activation [65].

Neuroinflammation, which plays a central role in SAE onset and development, can be produced by a reduction in the proportion and in the total perfused brain vessel density, as well as the alteration of the microcirculation [6, 22, 66, 67]. Notably, the main contributors to brain dysfunction are uncontrolled neuroinflammation and ischemic injury, which can cause the liberation of cytokines and able to activate the microglia [68]. Systemic inflammatory mediators produced by sepsis can enter the brain due to the BBB disturbance, causing the activation of microglia and neuroinflammation by releasing more pro-inflammatory cytokines [69]. The overproduction of pro-inflammatory cytokines can induce cholinergic neuron apoptosis, thus being reduced the cholinergic activity and the acetylcholine (ACh) neurotransmitter levels [69]. The reduction in cholinergic activity can lead to delirium and cognitive decline, characteristics of SAE. Furthermore, this reduction causes a decreased cholinergic inhibition of activated microglia, thus accelerating the microglia activation and, in turn, increasing the cytokine levels [69]. In addition, microglial activation causes hypothalamic neuroinflammation triggering neurologic alterations such as neurotransmission disturbances, as can be the release increment of glucocorticoid hormone or a cell death increment [6, 63]. This cascade leads to the immunosuppressive response, characteristic of sepsis and SAE, and exacerbates the infection worsening the outcome [69]. In the CNS, the cytokine increase is associated with infections, trauma, and injuries, among others [70, 71]. There is also an increase of transforming growth factor beta (TGF-β) and monocyte chemoattractant protein-1 (MCP-1) [72]. These mediators modify the expression of N-methyl-D-aspartate receptors (NMDARs) and α-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors (AMPARs) in neurons contributing to brain dysfunction [73]. Importantly, NMDARs and AMPARs are glutamate receptors. As it is known, glutamate is the most important excitatory neurotransmitter in the human brain, and when its levels are above the physiological range, excitotoxic effects are produced. This deleterious action of glutamate is induced in different types of brain insults, including neuroinflammation (for a recent review, see Joy and Carmichael [74]). Some studies have linked increased IL-1β levels with altered modulation of NMDARs, which may derive from functional disturbances in cognition and behavior and contribute to cognitive decline and depression in sepsis survivors [75–77]. It is also known that tryptophan, an AAA, is metabolized to quinolinic acid which can be synthesized in activated macrophages, acting as an excitatory transmitter stimulating NMDAR. The activation of these receptors can activate the neuronal isoform of the nitric oxide synthase (nNOS) and other calcium-dependent enzymes, releasing free radicals which can damage the DNA and activate the nuclear enzyme poly-ADP-ribose-synthetase (PARS), which its rapid activation depletes the intracellular concentration of NAD+, slowing the rate of glycolysis and ATP formation, so resulting in energy depletion, cell dysfunction, and death [78, 79]. These metabolic and molecular pathways are described in Fig. 2.

Role of miRNAs As a Potential Biomarker for SAE

microRNAs (miRNAs) are small noncoding RNAs which in recent years have been proposed as key biological regulators in many tissues and cell types, playing important roles in processes such as cell differentiation, growth, proliferation, apoptosis, metabolism, and cellular homeostasis [80–83]. miRNAs regulate the expression of many genes by linking their bases to complementary sequences of the 3′-untranslated region (3′-UTR), producing translation repression, or in 5′-UTR which stabilizes mRNA structure and facilitates its transcription [80, 84].

miRNAs have been postulated to play essential roles in normal brain functions and in many neuropathological conditions [85–87]. They are especially relevant in the brain because their small size miRNAs can cross the BBB easier than proteins or other biomolecules [88, 89]. When miRNAs are dysregulated, they can modify the expression levels of their mRNA targets, upregulating or downregulating gene expression and altering transcriptional programs, as demonstrated in many diseases, including sepsis [90, 91]. For that reason, the potential of miRNAs as biomarkers is obvious. Moreover, miRNAs have other features that make them optimal candidates as biomarkers, such as the fact they are present in biofluids, including blood, urine, and saliva, allowing relatively noninvasive sample collection [80, 92]. In addition to their accessibility, miRNAs are highly stable in biofluids and in different biospecimens making them relatively easy to work with and analyze using different methods (i.e., small RNA sequencing, arrays, qPCR, and ddPCR) [93–96].

Focusing on neurological inflammation, microglia have been postulated to play a central role in SAE since microglia mediates the immune response and the hyperinflammatory status in the brain [97, 98]. In this regard, microglia serves as brain-resident myeloid cells participating in cerebral development, ischemia, neurodegeneration, and neuro-viral

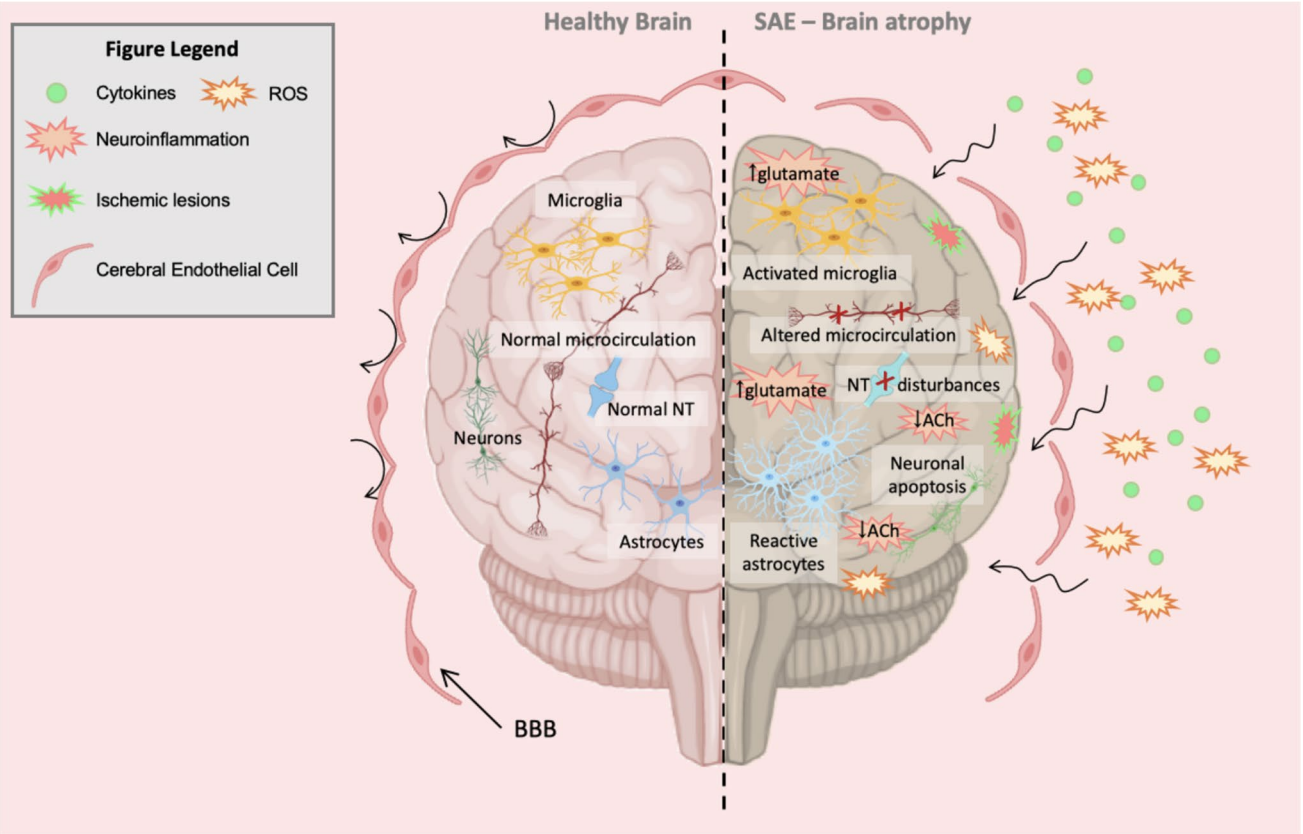

Fig. 2 Metabolic alterations during SAE pathogenesis. The main alterations observed in SAE physiopathology cause a hyperinflammation, which derives from microglial activation and brain dysfunction. At the molecular level, the NLRP3 inflammasome and the COX-2 pathways are activated, being inflammation the most relevant mechanism during SAE pathophysiology. SAE, sepsis-associated encephalopathy; BBB, blood–brain barrier; NLRP3, NLR family pyrin domain 3; ACh, acetylcholine; TNF-α, tumor necrosis factor alpha; IL, interleukin; NO, nitric oxide; ROS, reactive oxygen species; COX-2, cyclooxygenase-2; PGI_2, prostaglandin I2. Gray arrow indicates a cause; orange arrow indicates a release; green arrow indicates an activation; black line indicates an inhibition. Figure based on Chung et al. [46]

infection [51, 99]. In this scenario, miRNAs are one of the most important regulators mediating microglial activation, polarization, and autophagy and subsequently affecting neuroinflammation and the outcome of CNS disease [100–102].

Although very few miRNAs have been described in SAE, it is expected that some miRNAs could show a similar expression pattern that those observed in sepsis, especially miRNAs related to hyperinflammation. Moreover, other miRNAs related to brain damage can also have dysregulated levels, although they could not be specific for SAE.

Many circulating miRNAs have been associated with sepsis diagnosis and prognosis [103]. Puskarich et al. [91] observed a correlation between sepsis and inflamma-miRs such as miR-146a, miR-223, and miR-150 in plasma. Interestingly, miR-146a is involved in the regulatory T cells (Treg) survival and suppressor function [68], thus regulating immune response and targeting the tumor necrosis factor receptor-associated factor 6 (TRAF6), a ubiquitin-conjugating enzyme that mediates NF-κB activation [104]. Moreover, miR-223 directly participates in inflammation by targeting NF-κB [105]. NF-κB is closely related to inflammasome activation and therefore in pyroptosis, and importantly, it is upregulated in SAE [51]. Low levels of miR-223 and miR-146a were found in patients with severe sepsis [106], so we can suggest that low levels of miR-223 may contribute to maintaining high transcription of NF-κB. Regarding miR-150, its expression was correlated with mortality [91]. Moreover, Vasilescu et al. [107] also found that miR-486 and miR-182 expression was higher in septic patients than in healthy subjects. Importantly, some miRNAs such as miR-146a or miR-155 demonstrated their role in neuroinflammation. This is because both miRNAs regulate the overexpression and activation of NF-κB and therefore induce neural pyroptosis through activation of the IL-1β signaling pathway [51, 108, 109].

Notably, miR-155, miR-27a, and miR-210 have been widely postulated as biomarkers in sepsis, and recent studies showed that they play a central role in microglia function

[90, 110]. Moreover, some studies have shown that microglial cells are the front line target in the brain for lipopolysaccharide (LPS) action, reinforcing the idea of the key role of microglia in SAE [111]. Regarding miR-155, it was shown that its inhibition in microglia contributes to the development of endotoxin tolerance through an immunohomeostatic reaction [112]. This tolerance is caused by repeated exposure to LPS that maintains an altered response in immune cells, resulting in inhibition of the proinflammatory response and resolution of inflammation [112]. Since microglia is the first line of defense in the brain and the first cells which are the target of LPS in this tissue, it is expected that downregulation of miR-155 may protect microglia against LPS-induced inflammatory injury [111, 113, 114], which is frequent in septic infections. For that reason, it is expected to find high levels of miR-155 in SAE patients when bacterial LPS is found in septic patients. However, miR-155 is not specific, because it is also found at high levels in a wide variety of neurological diseases, for example, in Alzheimer's disease [88].

miR-27a was also found to be expressed in LPS-activated microglia, so it is possible that levels of miR-27a were reduced in SAE, postulating this miRNA as an interesting factor to be investigated in SAE. Moreover, miR-27a was able to inhibit microglia-produced inflammatory cytokines, including IL-6, IL-1β, and TNF-α, and blocking the expression of TLR4 and IL-1 receptor-associated kinase 4 (IRAK4) [115]. The capacity of miR-27a to regulate the expression of some key inflammatory mediators in microglia makes it a good candidate biomarker for SAE.

In addition, miR-210 is upregulated under hypoxic conditions; therefore, it is considered an important regulator of hypoxia response through the control of many functions such as DNA repair, mitochondrial respiration, angiogenesis, and cell proliferation [116, 117]. Likewise, low miR-210 levels have shown neuroprotective effects on mice with hypoxic-ischemic encephalopathy, due to its capacity for activating microglia, so it is upregulated during the development of the pathology [118–120]. Interestingly, miR-210 was related to ROS generation and inflammation in the brain through the ischemia–reperfusion process [119, 121]. Interestingly, due to ischemia–reperfusion injury is usually associated with SAE, miR-210 represents a good biomarker candidate to diagnose SAE. Moreover, targeting miR-210 is a promising approach to develop a miRNA-based therapy since it has been demonstrated that higher levels of this miRNA play a neuroprotective role [119].

Focusing on specific biomarkers for SAE, miR-370 is the most characterized biomarker associated with SAE. Visitchanakun et al. [122] demonstrated that mice with SAE had high levels of miR-370 in brain tissue, and the results were corroborated in plasma samples of SAE patients. This result suggests that miR-370 is very specific for SAE since it shows undetectable levels in patients with sepsis and other inflammatory diseases. Despite the specific role of miR-370 in SAE is not fully elucidated, some authors have postulated that miR-370 induces cell cycle arrest by targeting β-catenin that has a physiological role in controlling cell–cell adhesion and regulating gene transcription [123] and is able to inhibit the proliferation of human glioma cells [122].

In addition, some miRNAs postulated as key regulators in microglia have been shown to mediate inflammation in CNS pathologies [124]; therefore, they may play relevant roles in SAE pathology. In fact, transcriptome analyses comparing microglia, myeloid, and other immune cells identified 239 genes and 8 microRNAs that were highly expressed and specific for microglia: miR-29a, miR-29b, miR-342-3p, miR-103, miR-99a, miR-322, miR-125b-5p, and miR-30a [125].

Interestingly, miR-181b has been postulated to have a protective role in the hippocampus of septic rats. In fact, Dong et al. showed that the expression of hippocampal miR-181b was significantly decreased in septic rats. In this way, the upregulation of miR-181b can inhibit the activation of the NF-κβ signaling pathway and the release of the inflammatory cytokine IL-1β and TNF-α, which are elevated in plasma patients with SAE, therefore alleviating the inflammatory reaction and hippocampus injury in septic rats [126].

Another miRNA postulated as a regulator of microglia differentiation and inflammation was miR-101. Reiko et al. demonstrated that miR-101, which is enriched in the brain, regulates microglial morphology and inflammation, usually altered in SAE patients through the downregulation of the expression of MAPK phosphatase-1 [127]. Other authors showed that through the MAPK pathway, miR-101 also regulates cellular autophagy in the brain [128].

Another interesting miRNA is miR-203, which can inhibit ischemia induced by the activation of microglia, by targeting directly MyD88, a protein that plays a central role in the responses of microglia to pathogen-associated molecular patterns (PAMPs) through Toll-like receptors (TLRs) [90, 129]. Moreover, the overexpression of miR-203 in the brain induces the repression of NF-κβ signaling and prevents subsequent microglial activation ameliorating neuronal injury induced by hyperinflammation [130].

An important mechanism that induces an inflammatory state in CNS is the one mediated by inflammasomes. Some authors showed that the NLRP3-inflammasome complex plays an important role in sepsis through inflammation. NLRP3 inflammasome is considered one of the most important mechanisms that mediate the pro-inflammatory status in the early phases of sepsis [131, 132]. Importantly, it has been shown that NLRP3/caspase-1 pathway-induced pyroptosis mediates cognitive deficits in a mouse model of SAE [133]. Moreover, the inhibition of the NLRP3/IL-1β axis in the microglia improves spatial memory in mice with SAE [2]. Regarding miRNAs, Zhou et al. showed that miR-7 was able

to inhibit the activation of microglial NLRP3 inflammasome in vitro. Importantly, stereotactic injection of miR-7 mimics into the mouse striatum ameliorated microglial activation, concomitant with attenuation of dopaminergic neuron degeneration in a mouse model [134].

Similarly, Kumar and Nerurkar demonstrated that mice infected with West Nile virus showed differential expression of miR-196a, miR-202-3p, miR-449c, and miR-125a-3p in brain tissue, leading to neuroinflammation and neuronal death [135]. Furthermore, the upregulation of miR-32 was correlated with the levels of neuroinflammatory molecules [136]. In this regard, it has been demonstrated that the inhibition of miR-32 ameliorated inflammatory cytokine production in LPS-treated microglia, such as IL-1α, IL-1β, and NF-κβ pathway [88].

Finally, in a study performed by El-Assaad et al. [137], specific expression profiles of miRNA in the brain tissue after bacterial infection were found. They found that let-7i, miR-27a, miR-150, miR-126, miR-210, and miR-155 were differentially expressed in the brain. These findings suggest the possibility of measuring specific patterns of miRNA expression in the brain under bacterial infection, which may help to identify brain-related deleterious effects in sepsis. However, since miRNA expression profiles should not be specific for SAE, the use in combination with other biomarkers associated with sepsis may be an effective method

Table 1 miRNAs proposed as good biomarkers to diagnose SAE

miRNA	Role	Reference
miR-146a	It is involved in the suppressive and survival function of *Treg*, thus regulating the immune response. It plays its role through the regulation of TNF receptor-associated factor 6 (TRAF6), a ubiquitin-conjugating enzyme that mediates the activation of NF-κB. miR-146a was found upregulated in the plasma of septic patients	[57, 91]
miR-223	It participates in inflammation by targeting NF-κB expression. Low levels of miRNA-223 were found in patients with sepsis	[92, 93]
miR-150	miR-150 regulates cell differentiation fate in many hematopoietic cell lineages as T- and B-progenitor cells and NK-cells, among others. Its expression was correlated with mortality in septic patients	[90]
miR-155	miR-155 plays an essential role in neuroinflammation because it regulates the overexpression and activation of NF-κB and therefore induces neural pyroptosis through activation of the IL-1β signaling pathway Interestingly, its inhibition in microglia contributes to the development of endotoxin tolerance through an immune-homeostatic reaction. Therefore, it is expected that downregulation of miR-155 may protect microglia against LPS-induced inflammatory injury, which is frequent in septic infections	[39, 74, 95, 96, 98–101]
miR-27a	miR-27a is able to inhibit microglia-produced inflammatory cytokines, including IL-6, IL-1β, and TNF-α and block the expression of TLR4 and IRAK4	[102]
miR-210	miR-210 is an important regulator of hypoxia response through the control of many functions such as DNA repair, mitochondrial respiration, angiogenesis, and cell proliferation. Likewise, low miR-210 levels show neuroprotective effects on mice with hypoxic-ischemic encephalopathy, due to its capacity for activating microglia. Interestingly, miR-210 was related to ROS generation and inflammation in the brain	[91, 103–107]
miR-370	Despite the specific role of miR-370 in SAE is not fully elucidated, some authors have postulated that miR-370 induces cell cycle arrest by targeting β-catenin that controls cell–cell adhesion and regulates gene transcription. This protein is able to inhibit the proliferation of human glioma cells. miR-370 is the most and almost the only one characterized biomarker associated with SAE	[108, 109]
miR-181b	miR-181b has been postulated to have a protective role in the hippocampus of septic rats. The upregulation of miR-181b can inhibit the activation of the NF-κβ signaling pathway and the release of the inflammatory cytokine IL-1β and TNF-α, therefore alleviating the inflammatory reaction and hippocampus injury in septic rats	[112]
miRNA-101	miR-101 is a miRNA enriched in the brain that regulates microglial morphology and inflammation, usually altered in SAE patients through the downregulation of the expression of MAPK phosphatase-1. Other authors showed that through the MAPK pathway, miR-101 also regulates cellular autophagy in the brain	[113, 114]
miR-203	miR-203 can inhibit ischemia induced by the activation of microglia, targeting directly MyD88, a protein that plays a central role in the responses of microglia to PAMPs through TLRs. Moreover, the overexpression of miR-203 in the brain induces the repression of NF-κβ signaling, so it can prevent subsequent microglial activation ameliorating neuronal injury induced by hyperinflammation	[76, 115, 116]
miR-32	miR-32 acts an important role by inhibiting glioma cell proliferation. The upregulation of miR-32 was correlated with the levels of neuroinflammatory molecules and looks like a great candidate for SAE diagnosis	[122]

to diagnose SAE. Potential biomarkers for SAE based on miRNAs are summarized in Table 1.

Despite there are no optimal inclusion and exclusion criteria for SAE, it is difficult to apply a specific treatment. Some researchers are looking for good therapeutic strategies and drugs for SAE treatment; nevertheless, to perform a successful clinical trial, it is important to properly define inclusion and exclusion criteria [138]. Beyond the necessity of biomarkers to identify appropriate therapies, there is also a need to identify biomarkers to clearly define SAE. Some molecules that are being postulated for SAE treatment are showing promising results in animal models. Rocha Catalão et al. [49] showed that Simvastatin prevents long-term cognitive deficits in sepsis survivor rats, which is one of the main problems in sepsis and SAE survivor patients, by reducing neuroinflammation and neurodegeneration. They observed, in the hippocampus, a reduction of gliosis, nitrate, IL1-β, and IL-6 and overexpression of Bcl-2 protein levels, which was correlated with a decrease of apoptosis. Likewise, animals exposed to Simvastatin presented a better performance in tasks involving habituation, aversive and discriminative memory, and a reduction of neurodegeneration [49]. In another study, Zhang et al. demonstrated the ability of amitriptyline to reduce sepsis-induced brain damage. This compound works through the tropomyosin receptor kinase A (TrkA) signaling pathway, implicated in neuron survival and differentiation [139]. It has been demonstrated that amitriptyline is able to reduce cerebral inflammation by increasing the levels of IL-10, a potent anti-inflammatory cytokine, and reducing pro-inflammatory cytokine generation. Therefore, it is able to control gliosis and ROS production during the SAE physiopathology [139–141]. Another postulated therapy is pentamidine; Huang et al. [142] demonstrated the role of this antiprotozoal drug for inhibiting the S100B/RAGE/NF-κB signaling pathway, thus reducing neuroinflammation in the mouse hippocampus, attenuating ROS generation and gliosis.

Conclusions

SAE is a multifactorial syndrome categorized as a diffuse brain dysfunction with irreversible brain damage, which is associated with sepsis. SAE is the first organic dysfunction in many septic patients, and up to 70% of them can suffer from this syndrome. However, depending on the inclusion criteria, this percentage varies because there is no clear diagnostic method or biomarker to identify those patients. So the diagnosis of SAE represents one of the main challenges in SAE. Nowadays, molecular mechanisms underlying the SAE pathophysiology are not completely understood, but it seems that changes in brain metabolism, hyperinflammatory phenotypes, and alteration of the immune response play a central role in the SAE development and progression. Likewise, the microglia appear as a key player due to their function on the cerebral immune defense and their role in inflammation. miRNAs are involved in the brain immune response by regulating microglia, representing an effective form to assist clinicians to diagnose SAE and prognosticate SAE outcome, and helping to decrease high associated mortality. However, a single biomarker could be not enough to diagnose this heterogeneous syndrome, and a combination of biomarkers may improve the performance of the diagnostic methods. In this regard, some miRNAs have been postulated as biomarkers for SAE, such as inflamma-miRs and microglial-specific miRNAs. Specifically, circulating miR-370 was postulated as a feasible candidate for SAE diagnosis, alone or in combination with other miRNAs. Nevertheless, despite the advances in understanding SAE physiopathology, there is a clinically unmet need for the appropriate diagnosis of SAE. In this sense, identifying (bio)markers, single or in combination with other (diagnostic tools), with the potential to achieve a specific diagnostic of SAE, will improve patient care and reduce future morbidities in these patients.

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Declarations

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