Review

Biomarkers of Neuroinflammation and Epileptogenesis: A Systematic Review

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Biomarkers of Neuroinflammation and Epileptogenesis: A Systematic Review

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Abstract: Introduction: A central role for neuroinflammation in epileptogenesis has recently been suggested by several investigations. This systematic review explores the role of inflammatory mediators in epileptogenesis, its association with seizure severity, and its correlation with drug-resistant epilepsy (DRE). Material and Methods: The study analysed articles published in JCR journals from 2019 to 2024, including retrospective, prospective, case-control, or cross-sectional studies. It combined MESH and free terms for "Epileptogenesis" and "Neuroinflammation" and included searches for biomarkers previously reported in narrative reviews. Results: The study analysed 243 articles related to epileptogenesis and neuroinflammation, with 356 from selective searches by biomarker type. After eliminating duplicates, 324 articles were evaluated, with 272 excluded and 52 evaluated by authors. 21 articles were included in the qualitative evaluation, including 18 case-control studies, 2 case series, and 1 prospective study. This systematic review provides acceptable support for five biomarkers: TNF-a and some of its soluble receptors (sTNFr2), HMGB1 and TLR-4, CCL2 and IL-33. Discussions: Certain receptors, cytokines, and chemokines are examples of neuroinflammation-related biomarkers that may be crucial for the early diagnosis of refractory epilepsy or may be connected to the control levels of epileptic patients. Their value will be better defined by future studies.

Keywords: Epileptogenesis; Neuroinflammation; Biomarkers; HMGB1; TNF-a; TLR-4; sTNFr2; CCL2; IL-33; Drug Resistant Epilepsy

1. Introduction

Neuroinflammation refers to the inflammatory response of the Central Nervous System (CNS) to deviations from homeostasis that cannot be reversed by homeostatic mechanisms alone [1]. Today it is known that it is one of the pathophysiological processes that is transversally involved in multiple pathologies of the CNS [2]. Its role has been shown to be very relevant in the pathogenesis of diseases such as Multiple Sclerosis [3], the most paradigmatic example, but it is present in a wide variety of other diseases with special attention to neurodegenerative diseases [4,5]

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Residents innate immune cells (particularly microglia and astrocytes) are involved in this CNS inflammatory response, and cytokines and their receptors are involved, which act to promote the migration of leukocytes to the site of inflammation and endothelial adhesion. Understanding the processes that occur between the immune system and CNS is crucial, especially in the age of personalized medicine, since many of the elements identified can become diagnostic or prognostic biomarkers of the disease or even become therapeutic targets [6–8].

In recent years, several studies have addressed the role that neuroinflammation may play in epileptogenesis [9–11]. Although the molecular mechanisms underlying these pathophysiological processes are not yet fully understood, it has been speculated that inflammatory mediators may cause abnormal angiogenesis and impairment of permeability of the blood-brain barrier (BBB), a circumstance that is closely related to epileptogenesis [11,12]. On the other hand, the unregulated focal or systemic inflammatory processes themselves lead to the formation of aberrant neuronal connections and hyperexcitable neural networks as well as an altered response to neurotransmitters, thus participating in the process of epileptogenesis. For both reasons, over the past two decades there has been growing evidence of both clinical and basic studies providing strong support for the conclusion that neuroinflammation is involved in epileptogenesis [13–21].

Finally, the role of cytokines as potential pro-inflammatory mediators in the neuropathology of epilepsy may also contribute to elucidating the process of epileptogenesis [22–25]. Thus, the overexpression of some of these inflammatory mediators in the hippocampus and neocortex of patients with epilepsy indicates the activation of multiple pro- and anti-epileptogenic immune pathways.

We know that between 25 and 30% of epileptic patients are resistant to available anti-seizure medications (ASMs) [26] and some studies have shown that the levels of inflammatory mediators may be elevated in these patients [7,16,27]. This data could open the door to the use of these molecules as biomarkers that can be used as predictors of the inflammatory response and for the development of new treatments in drug-resistant epilepsy [28].

The purpose of this systematic review is to know the state of art in the comprehension of the role of inflammatory mediators in epileptogenesis, to know if elevated levels of inflammatory mediators in serum and CSF can be associated with seizure severity and recurrence, and to know which of them can be most correlated with DRE and could therefore be explored as biomarkers of DRE or epileptogenesis.

2. Methods

2.1. Search Strategy and Databases: A Systematic Search Was Carried Out Using the Following Inclusion Criteria

1. Articles published in journals indexed in JCR in the last 5 years (January 2019 to February 2024) using the MEDLINE database.
2. Articles that included retrospective, prospective, case-control or cross-sectional studies.
3. Searches were carried out by combining the following MESH and free terms: “Epileptogenesis” and “Neuroinflammation”
4. To obtain a more selective search for certain molecules of special interest, we included searches aimed at biomarkers that had previously been reported in narrative reviews of topic [11,22,29–32] also in combination with “Epilepsy” and “Epileptogenesis”

   Specifically, the molecules included in this additional search were:

- “High mobility group box 1/HMGB1”,
- “Toll-Like-Receptor 4/ TLR-4”,
- “Interleukin-1/IL-1”,
- “Interleukin-6/IL-6”,
- “Transforming growth factor beta/TGF-β” and
- “Tumour necrosis factor-alpha/TNF-α”.

5. Articles published in English and/or Spanish.

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The review protocol followed the declaration of Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA).

2.2. Exclusion Criteria

1. Duplicate articles, editorials, letters to the editor, or reviews (both narrative and systematics).
2. Articles that include basic research studies on tissues or animal models.
3. Articles on studies not related to any type of epilepsy or focused on acute symptomatic seizures due to infectious, traumatic, vascular or oncological processes.

2.3. Study Selection

Each of the selected articles was evaluated by reading the title and abstract and their keywords by two different reviewers (MJA and PSC) applying the eligibility and exclusion criteria mentioned above. In case of discrepancy between the two reviewers, the aid of a third reviewer (PCG) was needed. The selected articles were read in their entirety to evaluate the degree of scientific evidence. The included studies were assessed based on the criteria discussed in the Standards for Reporting of Diagnostic Accuracy (STARD) checklist [33].

2.4. Data Extraction

The required data from the inputs were extracted by one of the researchers (MJA) into Table 1. The level of the evidence was assessed using Scottish Intercollegiate Guidelines Network (SIGN) grading system [34].

Table 1. Summary of the studies included in the Systematic Review for qualitative analysis.

Reference	Biomarkers studied	Sample Type	Type of study	Results	N	Level of evidence
Kothur et al. [36]	IL-1ra, GM-CSF, IL-1β, TNF-α, IL-2,IL-4, IL-6, IL-8, IL-10, IL-13, IL-17A, IFN-γ, CCL2/MCP-1, CCL5/RANTES, CXCL1/GRO, CXCL10/IP-10,CCL3/MIP-1a, CCL4/MIP-1b, IL-12 (p40), IL-12 (p70), IFN-α, G-CSF, CCL11/eotaxin. IL-21, IL-23, CXCL13/BCA-1, CCL17/TARC, CCL21/6Ckine, CXCL12/SDF-1. CXCL9/MIG, CXCL11/I-TAC, and CCL19/MIP-3b	CSF	Case-control	TNF-α and CCL19 were mildly elevated in chronic epilepsy.	Patients with FIRES/FIRES-related disorders (FRD; n = 6), FSE (n = 8), afebrile status epilepticus (ASE; n = 8), and chronic epilepsy (n = 21)	2-
Yue et al. [46]	HMGB1 y TLR4	Serum	Case-control	HMGB1 y TLR4 were elevated in chronic epilepsy	72 epilepsy patients diagnosed with epilepsy vs 43 healthy controls	2
Jieun et al. [38]	α-synuclein, IFN-β, IFN-γ, IL-1β, IL-6, IL-10 and TNF-α	Serum	Case-control	α-synuclein levels were significantly increased in children with epilepsy.	115 epilepsy patients having afebrile seizure attacks within the last 48 h vs vs 146 healthy controls.	2+

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			Serum IL-1β levels showed significant correlation only with drug resistance in children with epilepsy.		
Saengow et al. [39]	gamma (IFN-c), IL-1β, and TNF-a	Serum	Case-control	IL-1β level was significantly decreased in patients with DRE. IFN-c level was significantly increased in patients with DRE. TNF-a showed no statistical change between groups.	65 patients with drug-resistant epilepsy vs 6 healthy controls	2-
Walker et al. [84]	HMGB1	Serum	Case-control	Patients with drug-resistant epilepsy had higher levels of HMGB1 than both healthy controls and patients with drug-responsive epilepsy	65 patients with drug-resistant epilepsy vs 74 healthy controls	2+
Kamaşak et al. [85]	HMGB-1, TLR-4, IL-1R1, TNF-a, IL-1β	Serum	Case-control	Significantly higher levels of HMGB-1, TLR-4, TNF-a and IL-1β in the severe epilepsy group than in the other two groups	28 children with DRE vs 29 children with controlled epilepsy vs 27 healthy controls	2
Aline et al. [86]	TNF-a, Caspase and Lipid factors	Serum	Case-control	No significant differences. But patients with generalized epilepsy demonstrated a significant correlation between TNF-α and caspase 8, caspase 3, and Picogreen.	43 epileptic patients vs 41 healthy controls	2
Alvim et al. [40]	IL-1, IL-2, IL-4, IL-6, IL-10, IL-17, IFNγ, TNF-α, soluble TNF receptor 1 (sTNFr1), sTNFr2, BDNF, neurotrophic factor 3 (NT3), NT4/5, ciliary neurotrophic factor (CNTF), nerve growth factor (NGF), and glial cell line-derived neurotrophic factor (GDNF).	Serum	Case-control	The plasma levels of BDNF, NT3, NGF, and sTNFr2 were higher, whereas IL-2, IL-4, IL-6, IL-10, IL-17, IFNγ, TNFα, CNTF, and sTNFr1 were lower in patients than controls. The molecule sTNFr2 was the best marker to discriminate patients from controls also differing between patients with frequent and infrequent seizures.	446 patients with epilepsy vs 166 healthy controls.	2+
Minchen et al. [47]	HMGB1 and TLR4	Serum	Case-control	HMGB1 and TLR4 levels were higher in epilepsy patients compared with	105 epilepsy patients vs 100 healthy controls	2

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				controls HMGB1 and TLR4 expressions were correlated with higher possibility of drugs resistance.		
Ethemoglu et al. [43].	IL-33	Serum	Case-control	IL-33 level was found higher in all the patients with epilepsy compared to the control group.	60 patients with epilepsy (21 patients with treatment-resistant epilepsy and 39 patients with well-controlled epilepsy) vs 35 control subjects	2
Panina et al. [51]	BDNF, TNF-*a*, HMGB1 and NTRK2	Serum	Case-control	A decrease in the concentration of BDNF, TNF-*a*, and HMGB1 was registered in the group of patients with TLE compared with the control group.	166 patients with epilepsy (49 with treatment-resistant epilepsy and 117 patients with well-controlled epilepsy) vs 203 controls.	2
Wang et al. [35]	IL-1β, IL-5, IL-6, IL-8, IL-17, IFN-γ and TNF-α	Serum	Prospective, population-based study	The level of TNF-α in the mTLE-HS-P group was significantly higher than that of the patients in the mTLE-HS-N and healthy control groups, and the level of TNF-α in the patients in the mTLE-HS-N group was significantly higher than that of the patients in the healthy control group.	71 patients with medial TLE vs 20 controls	2++
Milano et al. [41]	IL-6, TNF-α, IL-33, IL-8, CCL2, IL-13, IL-1β, IFN-γ, IL-1Ra, CCL3, IL-4, CCL4, IL-5, IL-1α, IL-17 A, IL-18, IL-33r, IL-1RII, IL-1RI	Serum	Case-control	Levels of CCL2, CCL3 and IL-8 were elevated in the serum of patients with epilepsy compared to healthy controls, without differences between drugs-resistant and drug-sensitive patients.	47 patients diagnosed with MTLE vs 25 healthy controls	2
Sokolova et al. [44]	IL-1RA, interferon IFN-, IL-10 IL-2, IL-8, IL-7, TNF-α, IL-4, sCD40L	Serum	Case-control.	The level of the immunoregulatory cytokine IL-2 and the chemoattractant proinflammatory IL-8 was decreased in DRE patients. Proinflammatory cytokines (TNF-α, IL-4, sCD40L) was increased.	6 DRE patients vs 5 healthy controls.	2-
Wang et al. [49]	HMGB1	CSF and Serum	Case-control	The CSF HMGB1 concentrations were significantly higher in the DRE vs the other groups. Patients with symptomatic etiology showed significantly	27 DRE patients, 56 Newly diagnosed epileptic patients and 22 other non-inflammatory neurological disorders	2-

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				high levels of CSF HMGB1. Patients without remission expressed elevated levels of CSF HMGB1 at one-year follow-up. CSF HMGB1 levels were positively associated with seizure frequency.		
Mochol et al. [87]	IL-18; Interleukine 18 binding protenin (IL-18BP)	Serum	Case-control	Increased serum levels of IL-18 and IL-18BP in epilepsy patients.	119 patients with epilepsy and 80 healthy controls	2
Nass et al. [50]	c-reactive protein (CRP), HMGB1, S100, RAGE, ICAM1 and MMP9	Serum	Case Series	Rapid postictal increase of HMGB1 and S100.	28 patients with Epilepsy with Generalized Seizures.	3
Gakharia et al. [52]	CCL2, CCL4 and CCL11 and PGE2	Serum	Case-control	High CCL11 and PGE2 levels correlated with their seizure frequency and epilepsy severity.	40 epileptic patients (20 DRE and 20 Controlled epilepsy) vs 16 healthy controls.	2
Bronisz et al. [53]	MMP-9, MMP-2, CCL-2, S100B, TIMP-1, TIMP-2, ICAM-1, TSP-2, P-selectin)	Serum	Case series	Levels of MMP-2, MMP-9, and CCL-2 were found to influence seizure count in 1, 3, 6, and 12 months of observation.	49 patients with epilepsy.	3
Gledhill et al. [37]	CRP, calbindin, cytokeratin-8, eotaxin, eotaxin-2, eotaxin-3, granulocyte-macrophage colony-stimulating factor, ICAM-1, IFN–γ, IL-1β, IL-1α, IL-2, IL-4, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12/IL-23 p40, IL-12 p70, IL-13, IL-15, IL-16, IL-17, IFN-γ-inducible protein 10, macrophage colony-stimulating factor (M-CSF), monocyte chemoattractant protein (MCP)–1, MCP-2, MCP-4, macrophage-derived chemokine, macrophage migration inhibitory factor, macrophage inflammatory protein (MIP)–1β, MIP-1α, MIP-5, matrix metalloproteinase	Serum	Case-control	TRAIL, ICAM-1, MCP-2, and TNF-r1 were elevated in epilepsy within 24 hours after seizure	137 patients with epilepsy vs 29 controls.	2+

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	(MMP)–1, MMP-3, MMP-9, Nectin-4, Osteoactivin, osteonectin, P-cadherin, serum amyloid protein A, stem cell factor (SCF), thymus and activation regulated chemokine, TNF–α, TNF-β, TNF–r1, TNF–r2 (R2), TNF–related apoptosis-inducing ligand (TRAIL), vascular cell adhesion molecule 1, and vascular endothelial growth factor A				
Česká et al. [42]	IL-6, IL-8, IL-10, IL-18, CXCL10/IP-10, CCL2/MCP-1, BLC, TNF-α, C-X3-X and fractalquine (CXC3CL1)	CSF and Serum	Case-control	Significant elevation of CCL2/MCP-1 in CSF and serum. Higher levels of fractalkine/CXC3CL1 in serum of pharmacoresistant patients than in controls	26 patients with epilepsy (22 DRE, 4 non-DRE) vs 9 healthy controls.	2-

2.5. Flowchart 2020 PRISMA

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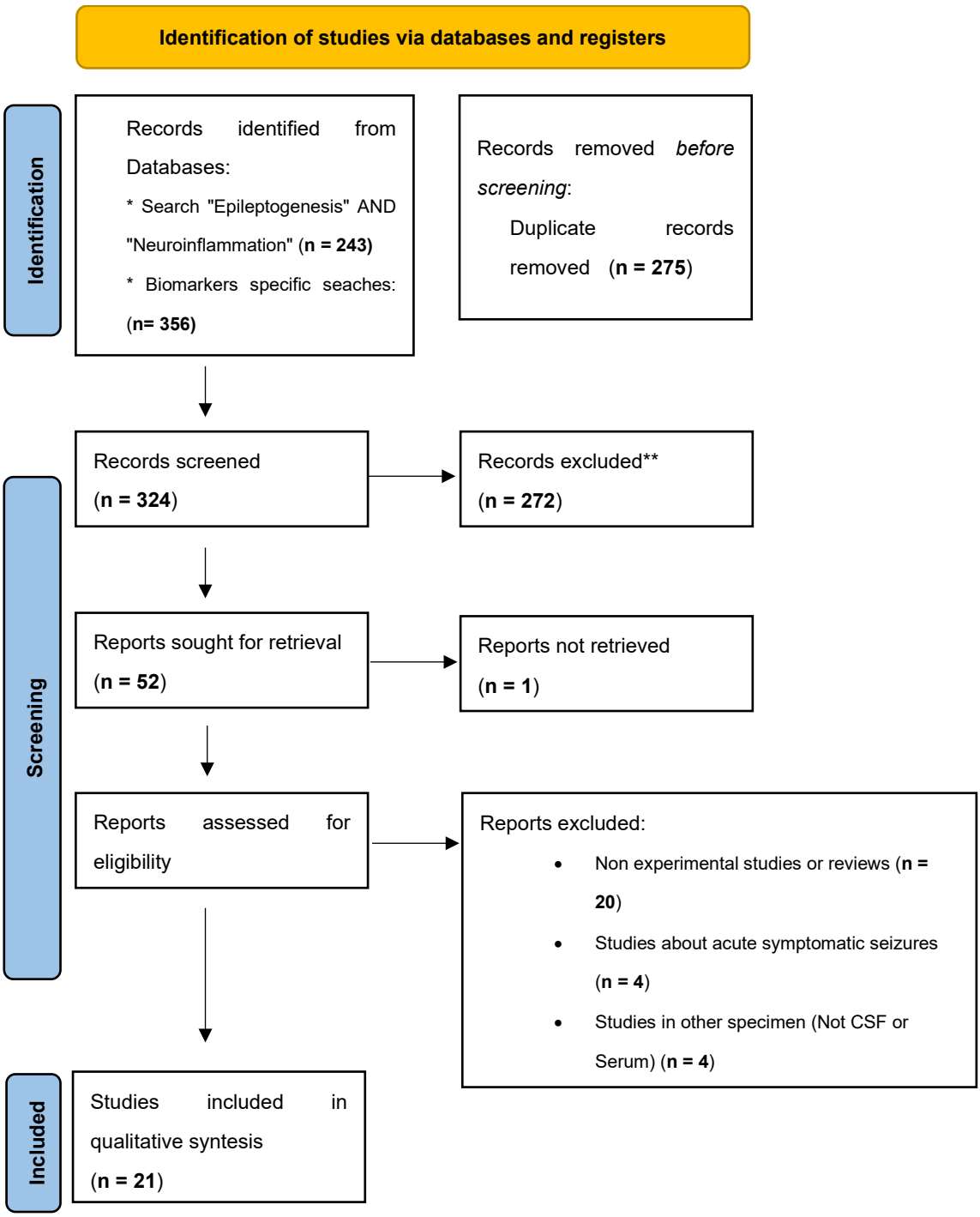

Figure 1. PRISMA 2020 Flowchart. *Consider, if feasible to do so, reporting the number of records identified from each database or register searched (rather than the total number across all databases/registers). **If automation tools were used, indicate how many records were excluded by a human and how many were excluded by automation tools. From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. doi: 10.1136/bmj.n71.

3. Results and Discussion

A total of 243 articles resulted from the search "epileptogenesis and neuroinflammation" and 356 from selective searches by biomarker type. Specifically, the results by type of biomarker were:

- HMGB1: 31 results
- TLR-4: 38 results

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- IL-1b: 69 results
- IL-6: 101 results
- TGF-β: 29 results
- TNF-a: 91 results

After eliminating duplicates, 324 articles were evaluated by reading their titles and abstracts and applying the defined inclusion and exclusion criteria. A total of 272 articles were excluded and another 52 were evaluated by the authors through reading the full text. Finally, 21 articles were included in the qualitative evaluation. See Figure 1 for PRISMA 2020 Flowchart. By type of articles, our selection included 18 case-control studies, 2 case series, and 1 prospective population-based study.

3.1. Interleukin 1β (IL-1β)

Our review has found studies with conflicting results. The one with the highest level of evidence is, a prospective study by Wang et al. [35] that does not show elevated levels of this biomarker in people with epilepsy. Other case-control studies also report similar results [36,37]. Choi et al. [38], conversely, in a retrospective case-control study found an elevation of serum and CSF IL-1β in children who had suffered epileptic seizures in the previous 48 h. Kamaşak et al.[30] studied 28 children with DRE of at least three years of evolution, demonstrating significant differences in IL-1β levels. Based on these results, it has been proposed as a biomarker of drug-resistance. Other studies, however, report decreased levels of this cytokine in DRE [39]. Our conclusion is that there is no conclusive evidence in this regard although it is possible that IL-1β could be a valid marker for the detection of recent seizures.

3.2. Interleukin 6 (IL-6)

We find 5 case-control studies [35,37,40–42] of which only one of them[40] found a decrease in serum levels in patients with epilepsies compared to healthy controls. For this reason, the role of this IL as a biomarker is not fully defined.

3.3. Interleukin 17 (IL-17)

A Prospective Study on patients with TLE [35] and 3 other case-control studies included in this systematic review [37,40,41] determined this IL but only the study by Alvin et al. [40] showed a decrease in their serum levels of patients with epilepsy compared to healthy controls. We consider that there is insufficient evidence to defend its role as a useful biomarker in clinical practice.

3.4. Other Interleukins

A significant additional number of ILs have eventually been studied, by case-control studies. We must highlight the study by Alvim et al. [40], which shows a decrease in serum levels of IL-2, IL-4, IL-6 or IL-10 in patients with epilepsy versus healthy controls. In the study by Ethemoglu et al. [43] IL-33 level was found higher in all the patients with epilepsy.

3.5. TNF-α

In total, 11 publications that are a part of our systematic review contain this molecule. Most of them did not demonstrate any appreciable distinctions between epilepsy sufferers and healthy controls. However, three of them—the TLE study by Wang et al. [35]., the Drug Resistant Epilepsy (DRE) study by Sokolova et al. [44]., and the chronic epilepsy study by Kothur et al. [36].—do indicate an increase in these levels in these individuals. According to this last study, there was an increase in CSF TNF-α during the acute stage of status epilepticus or FIRE-type encephalitis, and a decrease during the chronic phases.

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3.6. Transforming Growth Factor Beta (TGF-β)

Several investigations have been carried out to establish a connection between these experimental findings and drug-resistant epilepsy. In a case-control study, Yu et al. [45]. demonstrated that individuals with resistant epilepsy had higher TGFβ levels in their CSF than patients with controlled seizures. Since this article was published in 2014, it has not been incorporated into the systematic review. There are no other studies on this biomarker included in the Review, so we cannot prove its validity in patients with epilepsy.

3.7. Toll Like Receptor 4 (TLR-4)

Three of the case-control studies included in this review have demonstrated the association of high levels of this biomarker with drug-resistant epilepsy [30,46,47] as well as its correlation with the number of seizures [46]. So, it can be proposed as a biomarker not only of refractoriness but also of the control of epilepsy.

3.8. HMGB1

In our review we have found up to 6 case-control studies that have shown that HMGB1 is increased in the blood of patients with epilepsy compared to healthy individuals [46–50]. One of these studies analyses concomitantly the levels of this molecule in CSF and serum and finds elevation of its levels also in CSF but without correlation with serum values [49].

A further question some research address is whether the increase just happens immediately following a seizure or if it lasts for hours afterwards. Blood samples were taken by Nass et al. [50]. at the start of the investigation, as well as 2, 6, and 24 hours following a generalised tonic-clonic seizure. The findings verified that HMGB1 was raised right away and continued to be raised for six hours.

Some studies attempt to characterize this biomarker in DRE. So, Yue et al. [46]. found that serum HMGB1 levels were higher in patients with DRE compared to drug-responsive epilepsy. In addition, there was a positive correlation between HMGB1 expression and seizure frequency.

Walker et al. [48] discovered that DRE patients' serum had higher amounts of HMGB1 than did healthy controls and patients with drug-responsive epilepsy who had not experienced a seizure in at least six months. Still, no meaningful correlation was found with the epilepsy subtype or with the length or frequency of seizures during the preceding month. Kan et al. [47] also found a relationship between seizure frequency and duration.

Conflicting results were observed in only one study that was part of our review. Panina et al. [51]. discovered that, in contrast to a control group, patients with both controlled and refractory temporal lobe epilepsy had decreased serum HMGB1 levels. The authors contend that because their sample of patients had a wide range of epilepsy types and durations, their results are inconsistent with those of other studies.

These studies suggest that HMGB1 can be used as a biomarker of drug resistance in patients with epilepsy with a greater number of published studies supporting its use than other inflammatory markers included in this review.

3.9. Chemokines

In several studies of our review [36,41,42,52,53] some chemokines have been found to be increased. The one that has been identified in the largest number of studies is CCL2, a potent chemoattractant protein for monocytes, and hence is alternatively referred to as monocyte chemoattractant protein-1 (MCP-1). Elevated levels of CCL2 expression in an inflamed brain were associated with activation and recruitment of macrophages/microglia to the injury sites [54]. Česká et al. [42] compared cytokine levels in children with DRE with healthy control and control patients in both CSF and blood plasma, finding elevated levels of CCL2.

3.10. Soluble TNF-α Receptors

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According to the study of Alvim et al. [40], individuals with epilepsy had greater plasma levels of sTNFr2 than controls, but lower levels of TNFα and sTNFr1. Accordingly, sTNFr2 plasma levels are a promising indicator of epilepsy activity.

4. Discussion

Our systematic review's initial finding is that there isn't any research with a high degree of evidence. Out of the twenty-one selected articles, nineteen are case-control studies, while one is a prospective study [35], and one is an uncontrolled case series study [53]. Though two of them analyse CSF at the same time [42,49], the great majority concentrate on serum analysis and another only analyses CSF [36].

On the other hand, there is significant variation across the patient cohorts utilised in the various studies regarding the type of epilepsies included in them, the patient inclusion criteria, and the total number of patients employed. Because of this, there is a lot of variation in the findings, which makes comparing these researches challenging.

Cross-cutting phenomena such as neuroinflammation can arise in autoimmune, post-traumatic, viral, structural, or even hereditary epilepsies. The idea that neuroinflammation may be one of the triggers through a cascade of release of inflammatory and peripheral mediators is strongly supported by the data gathered over the past 20 years [10,11,55–57].

Our work serves to summarize the state of research as well as the potential for use in clinical practice of inflammatory biomarkers in plasma and CSF from patients with epilepsy and especially their relationship with DRE.

This systematic review provides acceptable support for five biomarkers: TNF-a and some of its soluble receptors (sTNFr2), HMGB1 and its TLR-4, CCL2 and IL-33 (Table 2).

Table 2. Summary of biomarkers that have shown some evidence in the systematic review without contradictory results.

Biomarkers	Number of studies with positive results	Sample	Quality of the evidence	Conclusions of the studies
HMGB1	6 Case-control studies	Serum & CSF	2+	Possible biomarker of DRE. Possible biomarker of seizure frequency. Temporal Relationship with Generalized Tonic-Clonic Seizures.
TNF-a	2 Case-control studies & 1 Prospective population-based study	Serum & CSF	2+	Possible biomarker of DRE.
TLR-4	3 Case-control studies	Serum	2	Possible biomarker of DRE. Possible biomarker of seizure frequency.
rTNFr2	1 Case-control study	Serum	2-	Possible biomarker of seizure frequency.
CCL2/MCP-1	1 Case-control study	Serum & CSF	2-	Possible biomarker of DRE

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IL-33	1 Case-control study	Serum	2-	Possible biomarker of epilepsy.

Certain autoimmune illnesses have been linked to increased amounts of the nuclear non-histone protein HMGB-1. Its two main receptors, TLR-4 and RAGE, mediate a biological process that stimulates inflammation [58] by starting the IL-1R/TLR-4 cascade, which produces some types of cytokines and raises the hyperexcitability of neurons [59]. Furthermore, because it stimulates the phosphorylation of the N-methyl-D-aspartate receptor (NMDAR) and raises the Ca2+ channel's permeability, the HMGB1-TLR-4 axis is essential for neuronal hyperexcitability. The breakdown of the blood-brain barrier (BBB) can also result from the activation of the HMGB1-TLR-4 axis, allowing the permeation of activated lymphocytes, antibodies, inflammatory cytokines, or albumin from peripheral blood to the CNS, aggravating inflammation. Elevated HMGB1 levels have been associated with several CNS disorders [60–63]. The results of our review position this biomarker as the most supported by recent studies, especially in the characterization of DRE [46,48] and as a biomarker of recent epileptic activity [50].

TLR-4 is an innate immune system receptor of HMGB1 y that has been proposed as a promoter of epileptogenesis [64]. The results of our systematic review allow us to propose this molecule as a biomarker of DRE.

Another molecule of interest in our work is the Pro-inflammatory cytokine TNF-α. This molecule is secreted by activated astrocytes and microglia; it raises microglial glutamate levels and promotes Ca2+ entry into the cell similarly to IL-1β. However, TNF-α also causes the GABA receptor to endocytose, which depletes membrane inhibitory receptors and has an inhibitory effect. These conflicting effects of TNF-α on excitatory and inhibitory receptors may be a factor in neuronal hyperexcitability and, consequently, epileptogenesis [25]. Among the cytokines most researched in relation to epilepsy is TNF-α. Our systematic review finds several studies that correlate this serum biomarker with DRE or chronic epilepsy [35,36,44].

Perhaps of greater interest, it has the identification of some of the TNF-a receptors as biomarkers. There are two types of TNFα receptors: membrane-bound (TNFr1 and TNFr2) and soluble (sTNFr1 and sTNFr2). Some studies suggest that sTNFr1 and sTNFr2 better reflect TNFα activity because they are more stable than TNFα. The role of TNFα in epileptogenesis is a matter of debate. Some authors have suggested a proconvulsive role for this molecule [11]. The results of our review corroborate that sTNFr2 could be a promising biomarker related to epileptic activity [65].

Astrocytes, microglia, and endothelial cells secrete a family of proteins known as chemokines. By controlling neurotransmitter-releasing, voltage-dependent, or G-protein-dependent channels, they modulate neuronal excitability and are crucial in the entry of immune cells into the brain via certain G-protein-coupled receptors (GPCRs) [66,67]. This accumulating evidence suggests that chemokines and downstream signalling pathways mediate the interaction between neuroinflammation and epileptogenesis. Some chemokines such as CCL2, CCL3, lCCL4, Fractalkine (CX3CL1), CXCL13, and the corresponding chemokine receptor CCR2, CCR5, C-X-C receptor 4 (CXCR4), CXCR5, have been found elevated in the hippocampus in animal models of epilepsy [68]. Elevated levels of CCL2 expression in an inflamed brain were associated with activation and recruitment of macrophages/microglia to the injury sites [54]. The results of our systematic review only allow us to propose CCL2 as a biomarker of epilepsy [41,42,53], a potent chemoattractant protein for monocytes, and hence is alternatively referred to as monocyte chemoattractant protein-1 (MCP-1).

Even if some of the other molecules under examination were preceded by encouraging experimental trials, there doesn't seem to be enough information about them at this moment to consider them as prospective biomarkers for persistent epilepsies.

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So, Il-1b is a cytokine produced by the resident cells of central innate immunity. The binding of IL-1β to its receptor (IL-1R) activates nuclear factor κB (NF-κB) and three mitogen-activated protein kinase (MAPK) signaling pathways, all of which are involved in the production of other cytokines and in the upregulation of genes related to inflammation and generation of reactive oxygen species. These signaling pathways lead to the activation of TLR-4 [32,69]. On the other hand, IL-1β influences the entry of calcium through the Nmethyl-Aspartate Receptor (NMDA), reduces glutamate uptake by astrocytes, and increases glutamate release by glial cells [70]. Both TLR-4 and IL-1β are expressed at low levels within the brain under basal conditions but can increase rapidly during acute pathological conditions. Thus, elevated levels of IL-1β have been found after febrile seizures [71] and its role in epileptogenesis has been postulated [72]. However, our systematic review has found contradictory results that prevent us from including this molecule as a consolidated biomarker in epileptogenesis right now. Further studies will be needed to decide the true role of this biomarker.

IL-6 plays a fundamental role in regulating the inflammatory response and activating adaptive immunity [73]. Astrocytes and neurons synthesize this substance, which can be released by perivascular and brain endothelial cells in response to inflammatory and infectious stimuli. Some older studies not included in our review suggested that IL-6 could be elevated in CSF and plasma immediately after tonic-clonic seizures [74,75] or in patients with focal epilepsies [76], but our systematic review fails to corroborate the results of these studies.

Of the rest of the Interleukins, only the study by Ozlem et al. [43] showed us that IL-33 level was found higher in all the patients with epilepsy. IL-33, a novel member of the cytokine family associated with IL-1 is widely expressed from a wide range of cell types and tissues, including astrocytes, neurons, microglia and oligodendrocytes.

Finally, TGF-b is involved in cell proliferation, growth, and differentiation. Its role in neuroinflammation, however, is still unclear. The TGFβ type I receptor is more highly expressed in the cytoplasm of astrocytes, according to research on neocortical temporal lobe tissue from thirty epileptic patients [77]. It has also been suggested that blocking this receptor in vivo reduces the likelihood of epileptogenesis [78]. Our systematic review does not allow us to propose it as a biomarker of epilepsy in view of the current evidence.

The role of inflammatory markers as biomarkers of epilepsy has already been discussed in previous revisions, such as Zhang et al. [64]. The connection between two signalling pathways, the HMGB1/TLR4 and IL-1β/IL-1R1 pathways and epilepsy is the main topic of this review. The study also looks at mediators' roles and their pathways of sequestration and acknowledges the need for more research on the possibility of cell-to-cell interference in neuroinflammation, including activated microglia, astrocytes, and neurons, as well as the function of pro-inflammatory substances like chemokines, cytokines, bioactive lipids and growth factors.

One of the main consequences of our study is the possible consideration of the TNF-a/sTNFr2 and HMGB1/TLR-4 axes as therapeutic targets in DRE. There is a previous systematic review on this topic [79]. Although the evidence found by these authors was also scarce, they conclude that the use of anti-IL-1, anti-IL-6, and anti-CD20 agents in patients with drug-resistant epilepsy and refractory status epilepticus has shown promising results and a good safety profile and that, concerning research perspectives, there is increasing interest in the potential use of anti-chemokine and anti-HMGB-1 agents. With regard to anti-TNF-a drugs, experience was limited to published case series in the treatment of Rasmussen's encephalitis with Adalimumab [80,81]. Indeed, there is an ongoing clinical trial trying to evaluate the benefit of Adalimumab in patients with Rasmussen Encephalitis (ClinicalTrials.gov Identifier: NCT04003922).

Glycyrrhizin, an HMGB1 inhibitor medication, was noted as a possible treatment agent in the narrative review our group [11] published on the same subject because it has shown neuroprotective and antiepileptic benefits in many animal models of epilepsy [82,83] Nevertheless, this medication has not been evaluated in epilepsy clinical studies.

5. Conclusions

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Despite the lack of strong scientific backing for the studies that are currently available, they do enable us to identify a number of neuroinflammation-related biomarkers that could be important in the early detection of DRE or could be connected to the level of control experienced by epilepsy patients. Some DAMP molecules like HMGB1 and its membrane receptors (TLR-4) are among them, as are cytokines like TNF-a and some of its soluble receptors like sTNFr2 or IL-33, as well as chemokines like CCL2. These biomarkers may occasionally be suggested as potential treatment targets for particular forms of epilepsy. Future research will enable us to better characterise its actual value.

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