Hindawi
Acta Neurologica Scandinavica
Volume 2023, Article ID 3030625, 9 pages
https://doi.org/10.1155/2023/3030625

Research Article

The Regulation of Plasma Interleukin-6 Levels Is Modified by Hippocampal Sclerosis and Its Lateralization in Drug-Resistant Temporal Lobe Epilepsy

Maria Peltola[1,2] **Pabitra Basnyat**[2] **Suvi Liimatainen**[3,4] **Sirpa Rainesalo**[5] **Marko Pesu**[6,7,8] **and Jukka Peltola**[2,3]

[1]*Department of Psychiatry, Tampere University Hospital, Tampere, Finland*
[2]*Department of Neurology, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland*
[3]*Department of Neurology, Tampere University Hospital, Tampere, Finland*
[4]*Administration Centre, Tampere University Hospital, Tampere, Finland*
[5]*Division 7, Emergency Department, Intensive Care and Anaesthesia, Tampere University Hospital, Tampere, Finland*
[6]*Immunoregulation, Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland*
[7]*Fimlab Laboratories, Tampere, Finland*
[8]*Gilead Sciences Finland Oy, Finland*

Correspondence should be addressed to Maria Peltola; maria.peltola@tuni.fi

Received 14 September 2022; Revised 4 January 2023; Accepted 9 March 2023; Published 14 April 2023

Academic Editor: Anna Estraneo

Background. Several studies have reported the association of the proinflammatory cytokine IL-6 with temporal lobe epilepsy (TLE), but the regulation of IL-6 in hippocampal sclerosis (HS) is less studied. *Objectives*. To analyse IL-6 levels and the IL-6/IL-10 ratio in a larger, well-characterized group of patients with drug-resistant focal epilepsy (DRE), focusing especially on TLE and TLE-associated HS. *Methods*. IL-6 levels were measured by ELISA in plasma in a cross-sectional cohort of 265 patients comprising TLE with HS ($n = 34$), TLE without HS ($n = 103$), extratemporal lobe epilepsy ($n = 92$), and idiopathic generalized epilepsy ($n = 36$). *Results*. The IL-6/IL-10 ratio was higher in TLE with HS than in TLE without HS (3.1 vs. 1.6, $p = 0.042$), whereas the median levels of IL-6 did not differ among epilepsy types. TLE without HS patients had a higher proportion of increased IL-6 levels than TLE with HS patients ($p = 0.021$). Additionally, IL-6 levels were higher in patients with right-sided HS than in those with left lateralization (1.71 vs. 0.80 pg/mL, $p = 0.04$). In TLE with HS patients, IL-6 levels showed a negative correlation with seizure frequency during the last month ($r = -0.342$; $p = 0.047$), whereas in TLE without HS patients, the correlation was positive but did not reach significance ($r = 0.136$; $p = 0.169$). *Conclusion*. IL-6 levels and the IL-6/IL-10 ratio are differentially regulated among patients with TLE depending on the presence of HS and its lateralization, suggesting that TLE with HS is a distinct category regarding cytokine activation.

1. Introduction

More than 30% of patients with epilepsy have inadequate seizure control with antiseizure medications (ASMs) fulfilling the criteria for drug-resistant epilepsy (DRE) [1, 2]. Accumulating data suggest a major involvement of inflammation in DRE [3]. During epileptogenesis, various factors can affect neuroinflammation such as the production of several inflammatory cytokines and chemokines by CNS-resident cells. These cells include activated microglia and astrocytes, neurons, macrophages, and B and T lymphocytes [4, 5].

Inflammation in the CNS is characterized by the upregulation of cytokines that can increase seizure susceptibility and may be involved in epileptogenesis [6]. On the other hand, seizure activity leads to the production of cytokines characterized mainly by high levels of proinflammatory cytokines such as interleukin (IL)-1β, IL-6, and tumour

necrosis factor (TNF)-α [7–9]. IL-6 is a pleiotropic cytokine involved in initiating, maintaining, and resolving of local and systemic inflammatory responses and plays an important role in the transition between acute and chronic inflammation [10]. In the CNS, IL-6 acts as an essential signalling molecule in various activities, ranging from neuronal physiology to neurodevelopment, neurotoxicity, and neuroprotection. Moreover, CNS-derived IL-6 can also stimulate the production of IL-6 in peripheral blood, which subsequently activates the acute phase reaction [7]. However, IL-6 can also exhibit a dual role in the CNS as a pro and anti-inflammatory cytokine, with several experimental studies supporting the neuroprotective role of IL-6 [11–13]. Conversely, IL-10 is an anti-inflammatory cytokine impacting the resolution of peripheral inflammation and is generally considered to be the essential immunosuppressive cytokine in the CNS, where IL-10 inhibits the production of proinflammatory cytokines by initiating several immunoregulatory mechanisms [14, 15].

Temporal lobe epilepsy (TLE) is the most common form of adult focal epilepsies and hippocampal sclerosis (HS) is a frequent aetiology in TLE. Hippocampal onset seizures account for at least 80% of all temporal lobe seizures, and most of the TLE patients with HS are resistant to ASMs; therefore, HS is regarded as one of the most clinically significant pathologies of DRE [16].

HS represents 36% of all focal epilepsy pathologies compared to other histopathological diagnoses, as reported in a recent large study [17]. The hippocampus is the most widely studied part of the brain in both human and experimental epilepsy. In addition, according to recent studies, HS in TLE is a distinct entity with multiple subtypes based on the histological patterns of subfield neuronal loss and gliosis in HS [16]. TLE with HS is considered a potentially progressive disorder with recurring seizures contributing to increased neuronal damage over time [18]. In focal epilepsy, accelerated progressive cortical thinning was demonstrated, especially among patients with HS and in patients with early onset of epilepsy [19].

We have previously reported increased serum levels of IL-6 in TLE patients compared to healthy controls and patients with extratemporal lobe epilepsy (XLE) [20, 21]. Recently, we detected chronically decreased plasma IL-10 in TLE patients with HS compared to those without HS. Moreover, we found that the seizure frequency and epilepsy duration can affect the IL-10 concentration in patients with epilepsy [22]. Due to IL-6 proconvulsive and IL-10 anticonvulsive features, we have demonstrated in DRE patients with deep brain stimulation (DBS) that the IL-6/IL-10 ratio was decreased over time following the initiation of DBS therapy, suggesting that the IL-6/IL-10 ratio may reflect the balance between the proconvulsive/neurotoxic and anticonvulsive/neuroprotective effects of these two cytokines [23].

In our previous study on a cohort of 91 patients with focal epilepsy, we did not investigate the influence of HS on IL-6 regulation [20]. Therefore, this study was undertaken to analyse IL-6 levels in a larger, well-characterized group of patients with drug-resistant focal epilepsy, focusing especially on TLE and TLE-associated HS. We also analysed the IL-6/IL-10 ratio in the same study group for whom IL-10 levels were measured previously [22].

2. Patients and Methods

2.1. Study Patients. This cross-sectional study included 265 consecutive adult patients with epilepsy treated at the Outpatient Clinic of Neurology of Tampere University Hospital. The study protocol was approved by the Ethics Committee of Tampere University Hospital, and all patients provided written informed consent according to the Declaration of Helsinki. Epilepsy was classified according to the International League Against Epilepsy (ILAE) guidelines [24]. Focal epilepsy types were categorized into TLE, frontal lobe (FLE), parietal lobe (PLE), occipital lobe (OLE), multifocal, or unknown focal epilepsies. If the patient had TLE, the patient was further designated to either the TLE with HS group or the TLE without HS group, depending on the presence of HS. The classification of epilepsy types was reevaluated for this study.

The definition of the epilepsy type was based on patient history, electroclinical findings (EEG/video-EEG), neuroimaging results, and aetiology. Most patients had undergone high-resolution brain MRI (1.5 Tesla) with a specific epilepsy protocol evaluated by a neuroradiologist. Data on the duration of epilepsy, epilepsy surgery, and all previous and current ASMs were collected. Drug-resistant focal epilepsy was defined as persistent seizures after administering two different ASMs with adequate dosing [25]. Patients with DRE in our study population were evaluated for the possibility of epilepsy surgery. Patients with moderate or severe mental retardation, dementia, or malignant brain tumour were excluded, but patients with chronic stable low-grade brain tumour were included. Patients with poststroke epilepsy were excluded due to a local practice according to which they are followed up by general practitioners [20]. Data regarding concomitant autoimmune diseases were collected. Blood sampling was done only if the patients did not have any signs or symptoms of acute infection.

2.2. Immunological Analyses. Blood samples were collected in a vacutainer EDTA vacuum tube and centrifuged at 3000 rpm for 10 minutes. The samples were stored frozen at −70°C until use. Plasma levels of IL-6 were determined using commercially available enzyme-linked immunosorbent assay (ELISA) kits according to the manufacturer's protocol (Pelikine Compact, Sanquin, Amsterdam, The Netherlands). The test sensitivity for IL-6 was 0.4 pg/mL. Plasma levels of IL-10 were determined as described in our previous publication [22]. Since the results of the IL-10 analysis for the current cohort were reported in our previous publication [22], we did not include the IL-10-related results in this study; however, we determined the IL-6/IL-10 ratio for this study.

The data of this study are available on reasonable request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

2.3. Statistical Analysis. The demographics and clinical characteristics of the patients are reported as the means and

Table 1: Demographics and clinical characteristics of patients.

Characteristics	TLE+HS	TLE-HS	XLE	IGE
n	34	103	92	36
F/M	22/12	46/57	47/45	22/14
Age, years	45 (18-69)	40 (17-76)	36 (17-70)	28 (16-56)
Age at onset, years	13 (1-42)	16 (0-59)	11 (0-60)	14 (4-49)
ILAE aetiology				
Structural	34 (100)	43 (41.7)	52 (56.5)	
Genetic				36 (100)
Immune		4 (3.9)	1 (1.1)	—
Infectious		8 (7.8)	3 (3.3)	—
Unknown		48 (46.6)	36 (39.1)	—
Duration of epilepsy, years	30.0 (4-57)	23.0 (2-70)	18.0 (0.4-55)	17.0 (1.4-40)
Seizure-free patients for 1 year before labs	2 (5.9)	16 (15.7)	13 (14.1)	14 (40.0)
At least one seizure 1 month before labs	26 (76.5)	68 (66.0)	60 (65.2)	14 (40.0)
Seizure frequency 1 month before labs	3.0 (0-10)	1.0 (0-60)	2 (0-95)	0.0 (0-5)
Autoimmune disease	4 (11.8)	10 (9.7)	6 (6.5)	2 (5.6)
Surgery				
No surgery	26 (76.5)	83 (81.4)	79 (85.9)	36
Epilepsy surgery	7 (20.6)	10 (9.8)	2 (2.2)	0
Other lesional surgery	1 (2.9)	9 (8.8)	11 (12.0)	0

Presented as the median (range) for age, age at onset, seizure frequency 1 month before labs, and the duration of epilepsy or the frequency and proportion (for the rest of the variables). Abbreviations: TLE+HS: temporal lobe epilepsy with hippocampal sclerosis; TLE-HS: temporal lobe epilepsy without hippocampal sclerosis; XLE: extratemporal lobe epilepsy; IGE: idiopathic generalized epilepsy; ILAE: International League Against Epilepsy. One patient with TLE-HS had missing information on surgery.

standard deviations (SDs) or the frequencies and proportions. To evaluate an association between the study groups and categorical variables, Pearson's Chi-square test, if assumptions were valid, or Fisher's exact test was used. Continuous variables were analysed using the nonparametric Kruskal–Wallis test or Mann–Whitney *U* test. Spearman's correlation coefficient test was used to evaluate the association between the cytokines and their ratio with the clinical characteristics of patients. All statistical analyses were performed using SPSS statistical software version 28.0.0.0 (SPSS Inc., Chicago, IL, USA). A p value ≤ 0.05 was considered significant. Figures were prepared using GraphPad Prism 5.02 software (GraphPad Software Inc., La Jolla, CA, USA).

3. Results

3.1. Clinical Data. A total of 265 patients with DRE (137 females and 128 males) with a median age of 38 years (range: 16-76 years) were included in the current study. The clinical characteristics of the patients are presented in Table 1. Altogether, 34 patients had MRI evidence of HS, including 20 patients with right lateralization and 13 patients with left lateralization; additionally, one had bilateral HS. Seven of 34 HS patients had MRI evidence of dual pathology: vascular malformation ($N = 3$), infection ($N = 1$), and cortical dysplasia (CD) ($N = 3$). Among 103 TLE-HS patients, 14 patients had CD, 4 had immune, 8 had infectious, 8 had trauma, 12 had tumour, 5 had vascular lesions, 3 had vascular malformation, and 49 had unknown aetiologies. Furthermore, we analysed IL-6 levels and the IL-6/IL-10 ratio in different aetiology groups based on epilepsy types, and we found no statistically significant difference between these groups either for IL-6 levels ($p = 0.079$) or the IL-6/IL-10 ratio ($p = 0.301$). Similarly, we found no statistically significant difference between the groups based on surgery either for IL-6 levels ($p = 0.197$) or for the IL-6/IL-10 ratio ($p = 0.068$).

Among TLE + HS patients, the prevalence of HS tended to be higher in females than in males (33.8% vs. 18.8% $p = 0.054$). Autoimmune diseases tended to be more common in patients with TLE, including both with and without HS, than in patients with non-TLE ($p = 0.070$). The median epilepsy duration was significantly longer in TLE+HS patients than in TLE-HS patients (30 vs. 23 years, $p = 0.010$), XLE (30.0 vs. 21.0 years, $p = 0.002$) patients, or IGE (30 vs. 16 years, $p < 0.001$) patients. Seizure frequency during the month before blood sampling was higher in TLE+HS patients than in TLE-HS, XLE, and IGE patients. Still, the difference was statistically significant only compared to IGE patients ($p < 0.001$).

3.2. Assessment of IL-6 Levels and IL-6/IL-10 Ratio

3.2.1. IL-6 Levels and IL-6/IL-10 Ratio Based on Epilepsy Types. In the whole study cohort, the median IL-6 level was 1.2 pg/ml (range 0.0–17.6 pg/ml), and the median IL-6/IL-10 ratio was 1.8 (range 0.0-130.4). The median levels of IL-6 did not differ among the epilepsy types (Figure 1(a)), whereas the IL-6/IL-10 ratio was higher in TLE + HS than in TLE-HS (3.1 vs. 1.6, $p = 0.042$, Figure 1(b)).

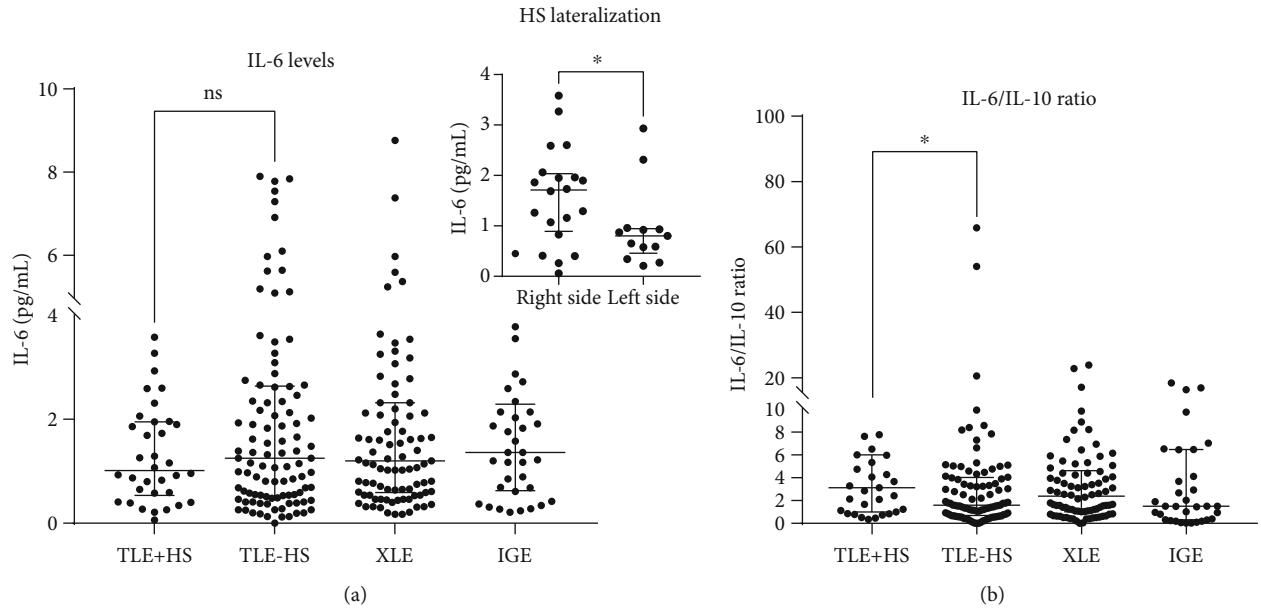

Figure 1: The distribution of IL-6 levels and the IL-6/IL-10 ratio based on epilepsy types. (a) IL-6 and (b) IL-6/IL-10 ratio. (a) The inset figure compares IL-6 between right and left lateralization of HS in TLE+HS epilepsy type. The scatterplot bars represent the median and interquartile range. Abbreviations: TLE+HS: temporal lobe epilepsy with hippocampal sclerosis; TLE-HS: temporal lobe epilepsy without hippocampal sclerosis; XLE: extratemporal lobe epilepsy; IGE: idiopathic generalized epilepsy.

Within the TLE+HS population, IL-6 median levels were higher in patients with right-sided HS than in patients with left lateralization (1.7 vs. 0.8 pg/mL, $p = 0.043$, Figure 1(a)), but the IL-6/IL-10 ratio did not differ between right and left lateralization ($p = 0.832$). The median (range) levels for IL-6 and the IL-6/IL-10 ratio for different epilepsy types are provided in Table 2. Furthermore, IL-6 and the IL-6/IL-10 ratio did not correlate with age, sex, or duration of epilepsy.

3.2.2. Categorical Assessment of IL-6 Levels in Different Epilepsy Types. Applying >5 pg/mL as a cut-off value for the increased levels of IL-6, as reported in our previous publication [20], 22 of 265 (8.3%) patients in the current study displayed elevated IL-6 levels. Out of 22 patients with increased levels, 14 had TLE (all TLE without HS), 7 had XLE, and one had IGE. Patients with TLE without HS had a significantly higher proportion of increased IL-6 levels than patients with TLE+HS ($p = 0.021$). The proportion of patients with increased IL-6 levels was higher in TLE without HS than in XLE and IGE, but the difference did not reach statistical significance. The frequency and proportions of patients with increased IL-6 levels in all epilepsy types are provided in Table 3.

3.2.3. IL-6 and IL-6/IL-10 Ratio and Seizure Frequency. Neither IL-6 levels nor the IL-6/IL-10 ratio differed between 45 seizure-free patients and 218 patients with seizures during the year before the study ($p > 0.05$). In a similar analysis among patients with or without seizures during the last month before the study, IL-6 levels and the IL-6/IL-10 ratio did not differ between the two groups ($p > 0.05$). Based on epilepsy types, there were no differences in either IL-6 levels or the IL-6/IL-10 ratio between seizure-free patients and patients who had seizures during the last year ($p > 0.05$).

Table 2: IL-6 levels and IL-6/IL-10 ratio based on epilepsy types and HS lateralization.

Epilepsy types	Median (pg/mL)	IQR
IL-6		
TLE + HS	1.0	0.5-1.9
Right lateralization	1.7	0.9-2.0
Left lateralization	0.8	0.5-0.9
Bilateral	0.3	NA
TLE-HS	1.2	0.5-2.6
XLE	1.2	0.6-2.4
IGE	1.3	0.6-2.2
IL-6/IL-10 ratio		
TLE + HS	3.1	1.0-6.0
Right lateralization	2.9	0.8-7.6
Left lateralization	3.6	1.0-5.3
Bilateral	3.1	NA
TLE-HS	1.6	0.7-4.0
XLE	2.4	1.0-4.8
IGE	1.5	0.3-6.5

Abbreviations: TLE+HS: temporal lobe epilepsy with hippocampal sclerosis; TLE-HS: temporal lobe epilepsy without hippocampal sclerosis; XLE: extratemporal lobe epilepsy; IGE: idiopathic generalized epilepsy; IQR: interquartile range (25^{th}-75^{th} percentiles); NA: not applicable.

Correlation analyses were performed to explore the association of IL-6 and the IL-6/IL-10 ratio with seizure frequencies (one year before and one month before the lab). In the whole cohort, IL-6 and the IL-6/IL-10 ratio did not correlate with seizure frequencies. In TLE with HS patients, IL-6 levels showed a significant negative correlation with the frequency of seizures during the last month before the lab ($r = -0.342$;

Table 3: Frequency and proportion of patients with increased IL-6 levels.

IL-6 levels (cut-off 5 pg/mL)	TLE+HS	TLE-HS	XLE	IGE	p value
Increased	0	14 (13.6%)	7 (7.6%)	1 (2.8%)	0.039
Not increased	34 (100%)	89 (86.4%)	85 (92.4%)	35 (97.2%)	

Abbreviations: TLE+HS: temporal lobe epilepsy with hippocampal sclerosis; TLE-HS: temporal lobe epilepsy without hippocampal sclerosis; XLE: extratemporal lobe epilepsy; IGE: idiopathic generalized epilepsy.

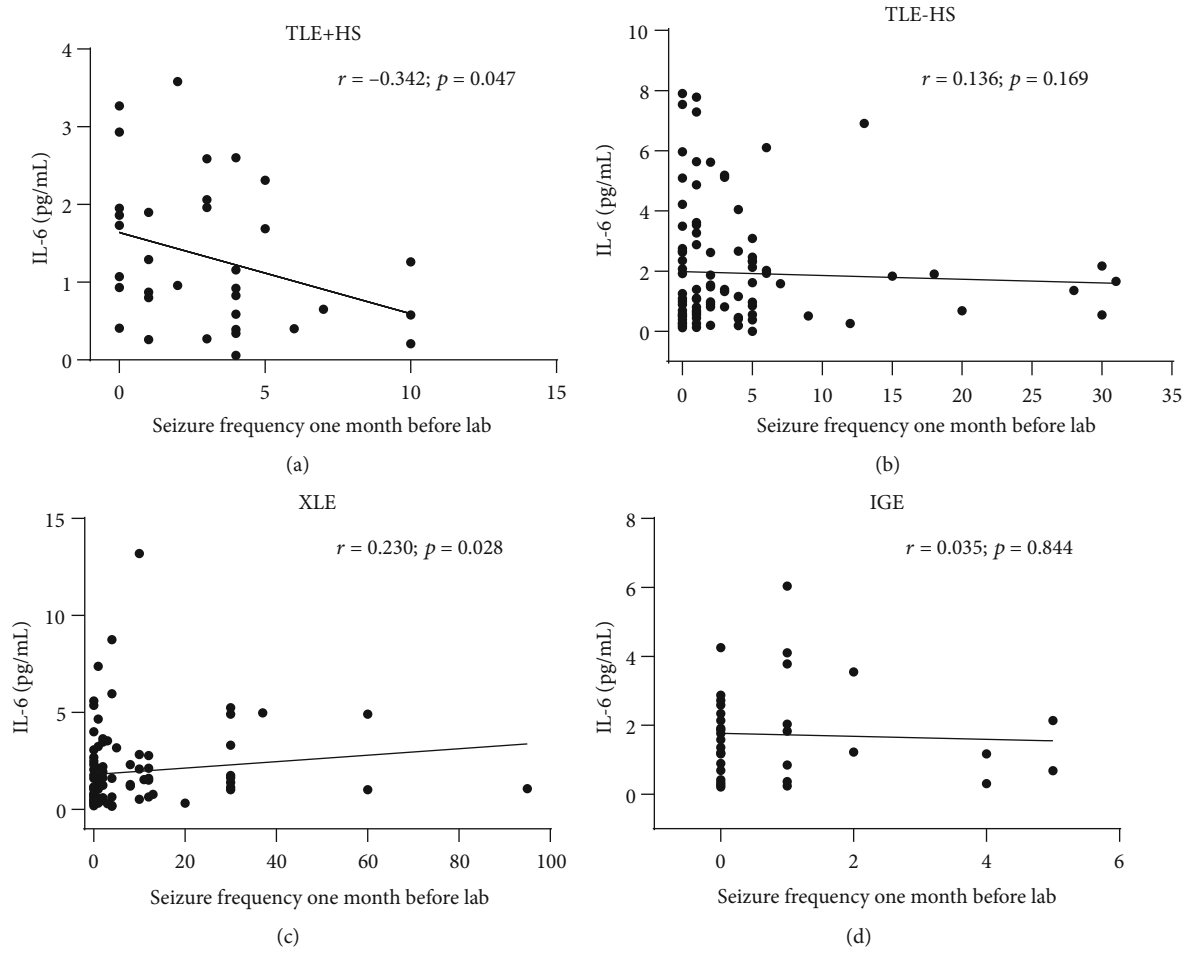

Figure 2: Spearman's correlation analysis between IL-6 levels in plasma and seizure frequency one month before the lab in patients with different epilepsy types. Abbreviations: TLE+HS: temporal lobe epilepsy with hippocampal sclerosis; TLE-HS: temporal lobe epilepsy without hippocampal sclerosis; XLE: extratemporal lobe epilepsy; IGE: idiopathic generalized epilepsy.

$p = 0.047$, Figure 2(a)). However, in TLE-HS patients, the correlation was positive without reaching significance ($r = 0.136$; $p = 0.169$, Figure 2(b)). Similarly, in XLE patients, IL-6 levels ($r = 0.230$; $p = 0.028$, Figure 2(c)) and the IL-6/IL-10 ratio ($r = 0.199$; $p = 0.064$) both displayed a positive correlation with seizure frequency one month before the lab, but statistical significance was achieved only for the IL-6 levels. In IGE patients, neither IL-6 ($r = 0.035$; $p = 0.884$, Figure 2(d)) nor the IL-6/IL-10 ratio correlated with seizure frequency.

In addition, we performed a separate correlation analysis between IL-6 and seizure frequency one month before the lab in TLE+HS patients based on lateralization. The results showed that the strength of the negative correlation was greater in patients with left lateralization ($r = -0.546$; $p = 0.053$) than in patients with right lateralization ($r = -0.197$; $p = 0.405$) (Supplementary Figure 1).

3.2.4. IL-6 Levels and IL-6/IL-10 Ratio according to Aetiology with a Focus on HS with Dual Pathology. IL-6 levels and the IL-6/IL-10 ratio did not differ between different aetiologies ($p > 0.05$). In descriptive analyses among all aetiology groups, five patients with immune aetiology showed the highest median levels of IL-6 (median = 3.0 pg/mL), whereas 10 patients with infectious aetiology had the highest median IL-6/IL-10 ratio (median = 3.3). The classification of aetiologies and the corresponding IL-6 levels and IL-6/IL-10 ratio are provided in Figures 3(a) and 3(b).

Next, we compared IL-6 levels and the IL-6/IL-10 ratio in HS patients with dual pathology. When comparing IL-6

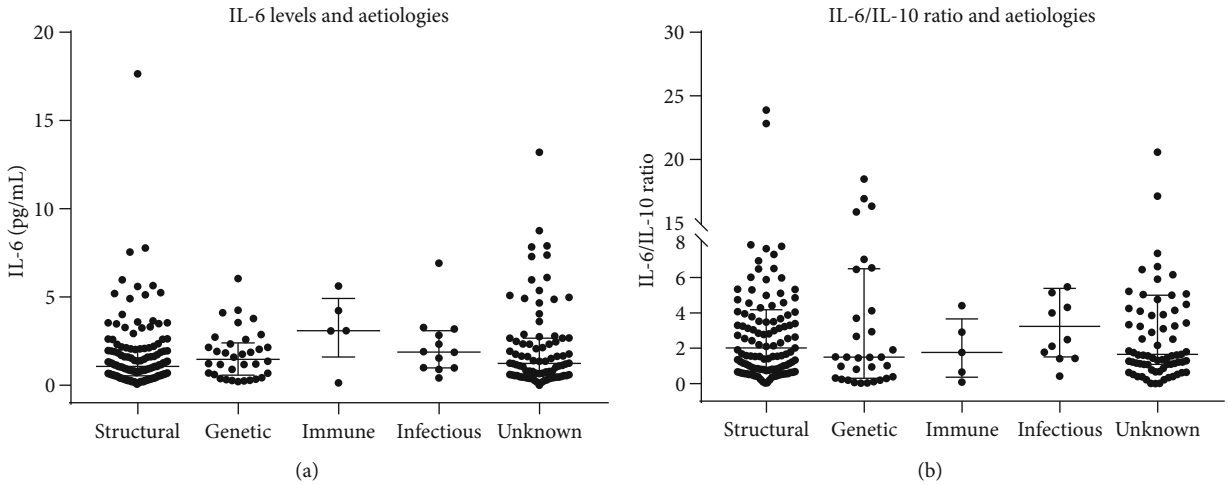

Figure 3: The distribution of (a) IL-6 and (b) the IL-6/IL-10 ratio based on aetiologies. The scatterplot bars represent the median and interquartile range.

levels in patients with dual pathology to CD- and HS-only patients, patients with CD+HS ($n = 3$) had higher median levels of IL-6 compared to HS only ($n = 27$) (2.6 vs. 1.0 pg/mL) or CD only (2.6 vs. 1.3 pg/mL) but lacked a statistically significant difference ($p > 0.05$). However, the IL-6/IL-10 ratio was significantly higher in the CD+HS group than in the HS group (7.7 vs. 2.6, $p = 0.036$) or CD alone (7.7 vs. 2.2, $p = 0.014$).

3.2.5. Effect of Autoimmune Diseases on IL-6 and the IL-6/IL-10 Ratio. In the whole cohort, patients with autoimmune diseases tended to have higher IL-6 levels than patients without autoimmune diseases [median range: 1.8 (0.1-7.2) vs. 1.2 (0.0-17.6) pg/mL, $p = 0.056$]. Such a difference in IL-6 levels was not observed when analysing between epilepsy types with or without autoimmune diseases. Similarly, there was no effect of autoimmune diseases on the IL6/IL-10 ratio when analysed in the whole cohort or among the epilepsy types.

4. Discussion

The present study provided new perspectives on immunological alterations in patients with DRE. First, the IL-6 concentration and proinflammatory IL-6/anti-inflammatory IL-10 ratio were differentially regulated among patients with TLE, depending on the presence of HS. Second, we present confirmatory evidence of the robust effect of the lateralization of HS on IL-6 levels. As far as we know, this is the first report that in TLE+HS patients, IL-6 levels displayed a statistically significant negative correlation with the frequency of seizures during the last month before the lab. In contrast, there was a positive correlation in patients with TLE and XLE. On the other hand, in the current study, we did not detect any correlation of the IL-6 or IL-6/IL-10 ratio with age, sex, or duration of epilepsy, as reported in previous studies [20, 26, 27].

According to our study, TLE without HS patients had a higher proportion of increased IL-6 levels than TLE with HS patients, whereas median levels of IL-6 did not differ among epilepsy types. The overall prevalence of increased levels of IL-6 in the present study is comparable to our previous study. However, in terms of comparison between TLE and XLE, the results from the present study are in contradiction to our previous study, where all patients with increased levels of IL-6 had TLE, and the median levels of IL-6 were also higher in the patients with TLE than in the patients with XLE [20]. This discrepancy could be due to the difference in the patient population with different clinical parameters. For instance, the epilepsy duration of TLE and XLE patients was similar in our previous study, but TLE-HS patients had longer disease duration than XLE patients in the present study. Also, seizure parameters that were evaluated in the current study—seizure-free patients for 1 year before labs, seizure frequency 1 month before labs, and patients having at least one seizure 1 month before labs, were all similar between TLE and XLE patients. This may partly explain the absence of seizure-induced elevation of IL-6 between these two groups. Since TLE patients are mostly drug resistant to ASMs, the higher levels of cytokines in these patients are due to some active epileptic processes occurring in the brain but such observation could not be depicted in this cohort. Moreover, we could not rule out the role of proinflammatory cytokines other than IL-6 in this study or the role of other cellular factors in different epilepsy types.

Similar to our previous study, findings from a recent meta-analysis also reported elevated serum IL-6 levels in TLE patients [28]. On the other hand, in line with our present result, a study analysing 14 serum cytokines, including IL-6 and IL-10, in a large number of epileptic patients ($n = 1218$) found no statistically significant difference in interictal cytokine levels among patients among TLE, XLE, and IGE groups [29]. Furthermore, in a recent study by Alvim et al. [26], the levels of IL-6 and IL-10 did not differ between epilepsy types.

The finding that the IL-6/IL-10 ratio was significantly higher in TLE patients with HS than in TLE patients without HS supports the importance of HS in TLE in our study. So far, this may be the first study reporting the IL-6/IL-10 ratio

in different epilepsy types. Not only a single cytokine measurement but also cytokine ratios have been studied in epilepsy because these ratios can more appropriately capture the intensity of inflammation versus the adequacy of the anti-inflammatory response [30]. Cytokine ratios have been studied in relation to either as a protective role for controlling seizures or as a mediator of the development of seizures. For instance, IL-1RA/IL-6 ratio was proposed as a potential biomarker for the development of hippocampal injury following febrile status epilepticus in children [30]. Recently, we measured plasma levels of IL-6 and IL-10 and their ratio in DRE patients, who were treated with deep brain stimulation of the anterior nucleus of the thalamus (ANT-DBS), and evaluated the changes in cytokine levels and their ratio during a 1-year follow-up [23]. We found out that the higher pretreatment levels of IL-6 and IL-6/IL-10 ratio subsequently decreased over time following the DBS therapy, whereas the levels of IL-10 levels increased over time following treatment with ANT-DBS. Therefore, we suggested that cytokines IL-6 and IL-10 and their IL-6/IL-10 ratio could have biomarker potential to evaluate the therapeutic response to DBS treatment, which could facilitate treatment optimization in patients with DRE.

Cytokine regulation is a hallmark of systemic inflammation. A significantly higher IL-6/IL-10 ratio in TLE+HS may reflect the dysregulation in proinflammatory and anti-inflammatory cytokine balance, a possible pathophysiological mechanism contributing to neuroinflammation in DRE [31]. Most TLE-associated HS studies on cytokines, particularly IL-6 and IL-10, are performed in HS tissue samples rather than in body fluids, plasma, or serum samples, providing inconsistent results [32–35]. A recent study found an upregulated RNA tissue expression of several immune mediators such as IL-1β, IL-18, CCL2, CCL3, and CCL4 in TLE with HS patients when compared to the postmortem hippocampal samples collected from autopsy controls suggesting their role in the neuroinflammatory process contributing to mesial TLE epileptogenesis [32]. However, it is interesting that the authors did not find detectable expression of IL-6 either in the TLE patients with HS or in the autopsy controls [32]. It is complicated to compare our results of systemic levels of IL-6 with the brain tissue expression of other cytokines because RNA tissue expression and plasma protein levels are not directly comparable; moreover, the increased cytokines tissue expression in the brain, as epilepsy-associated changes, may not be directly observed in the systemic circulation.

IL-6 levels were significantly higher in HS patients with right lateralization than in those with left lateralization, suggesting the lateralized cerebral influence of immune processes. It has been reported that the left and right cerebral hemispheres play a differential role in immune functions in humans [36]. Furthermore, in agreement with our findings, the study reported higher IL-6 levels after right-sided seizures than after left-sided seizures, suggesting the pivotal role of lateralization in seizure-induced production of IL-6 [37]. Cerebral hemispheres function differently in neurological and psychiatric disorders as well as immunity. Meador et al., [36] had proposed the cerebral lateralization of immune functions. It is suggested that the left hemisphere damage can result in the depression of immunological entities such as proliferation of T-lymphocytes, activity of natural killer cells, and the production of cytokines such as IL-2 and antibodies such as immunoglobulin G antibodies. Right hemisphere damage can induce either no immunological alterations or even augments the activity of specific immunological components [38]. Patients with epilepsy surgery displayed a differential role of the left and right cerebral hemispheres on immune functions; left resection patients induced decreased total lymphocytes, T cells, CD8, and CD4 after resection surgery, whereas for the patients with right-sided resection, the findings were opposite [36]. Moreover, an experimental study had revealed that the stimulation of the left temporo–parieto–occipital cortex temporarily increased thymic CD4+ and CD8+ lymphocyte production, whilst stimulation of the right hemisphere decreased their levels [39]. In a systematic review article by Sumner et al. [38], of 11 selected studies, three reported an association between weaker function of the left hemisphere and poorer immune function, three suggested an association between weaker function of the right hemisphere and enhanced immune functioning, and five described both associations.

Notably, we found a negative correlation of IL-6 levels with the frequency of seizures in TLE with HS suggesting that seizure-induced production of IL-6 was not observed in TLE with HS patients. This finding also implicates that seizure-induced production of IL-6 is dependent on the epilepsy types because we observed positive correlations among patients with other epilepsy types, that is, in TLE without HS and XLE patients. A similar observation was reported in a previous study where an increase in IL-6 levels after the seizure was lacking in patients with HS compared to patients without HS [37]. Seizure-induced IL-6 production is well established in epilepsy and can be apprehended only up to 24 hours after the seizure and then returns to a basal level; frequent seizures are usually associated with high levels of cytokines [37, 40]. We have previously reported an association between the levels of IL-6 and the severity of seizures in DRE patients with focal epilepsy [41, 42] and that the production of IL-6 as a response to a single seizure in TLE was dependent on the previous seizure frequency and the baseline IL-6 levels [21]. However, these studies were unable to explore the association of IL-6 in TLE patients with HS.

There are some limitations in the present study. The lack of a healthy control group is a crucial limitation of this study. Measurement of IL-6 in a healthy population would have provided additional findings enhancing the significance of this study. For example, we could have compared physiological concentrations of IL-6 in healthy controls with IL-6 levels in patients with different epilepsy types. We determined only a single cytokine at a single time point, and the additional measurement of some proinflammatory cytokines in follow-up samples could have enabled us to achieve a better picture of cytokine alterations in patients with HS. Moreover, the heterogeneity in aetiologies, epilepsy syndrome, seizure frequency, and disease duration limited the statistical power in groups with a small number of patients. The

evaluation of hippocampal structures was categorical in the present study without volumetric analyses of the hippocampus and the total brain volume.

Furthermore, IL-6 pathway has emerged as a pivotal pathway both for immune regulation in health and for dysregulation in many infective and rheumatic diseases [12]. In addition, recent exploratory studies have suggested that IL-6 levels are elevated also in cases of COVID-19 infection [43]. In our study, blood sampling was done only if the patients did not have any signs or symptoms of acute infection; in addition, this study was performed prior to COVID-19 pandemic.

5. Conclusions

In summary, our study provides evidence that IL-6 levels and the IL-6/IL-10 ratio are distinctly regulated among patients with TLE depending on the presence of HS and its lateralization, suggesting the lateralized cerebral influence of immune processes in patients with TLE. Moreover, the IL-6/IL-10 ratio was associated with TLE with HS as a reflection of one of the pathophysiological mechanisms contributing to neuroinflammation in HS. The influence of seizure frequency on the production of IL-6 depends on the epilepsy type. The significance of these new findings needs to be investigated further for their clinical relevance in TLE patients with hippocampal sclerosis with better MRI characterization of subtypes of HS.

Data Availability

The data of this study are available on reasonable request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Conflicts of Interest

The authors declare that there are no conflicts of interest regarding the publication of this paper.

Acknowledgments

The authors thank the patients who participated in this study.

Supplementary Materials

Supplementary Figure 1: Spearman's correlation analysis between IL-6 and seizure frequency one month before the lab in TLE + HS patients based on lateralization. *(Supplementary Materials)*

References

[1] P. Kwan, A. Arzimanoglou, A. T. Berg et al., "Definition of drug resistant epilepsy: consensus proposal by the ad hoc task force of the ILAE commission on therapeutic strategies," *Epilepsia*, vol. 51, no. 6, pp. 1069–1077, 2010.

[2] P. Kwan and M. J. Brodie, "Early identification of refractory epilepsy," *The New England Journal of Medicine*, vol. 342, no. 5, pp. 314–319, 2000.

[3] A. Vezzani, "Brain inflammation and seizures: evolving concepts and new findings in the last 2 decades," *Epilepsy Currents*, vol. 20, Supplement 6, pp. 40S–43S, 2020.

[4] A. Vezzani, S. Balosso, and T. Ravizza, "Neuroinflammatory pathways as treatment targets and biomarkers in epilepsy," *Nature Reviews Neurology*, vol. 15, no. 8, pp. 459–472, 2019.

[5] D. N. Xanthos and J. Sandkühler, "Neurogenic neuroinflammation: inflammatory CNS reactions in response to neuronal activity," *Nature Reviews Neuroscience*, vol. 15, no. 1, pp. 43–53, 2014.

[6] M. A. Galic, K. Riazi, and Q. J. Pittman, "Cytokines and brain excitability," *Frontiers in Neuroendocrinology*, vol. 33, no. 1, pp. 116–125, 2012.

[7] J. Bauer, A. J. Becker, W. Elyaman et al., "Innate and adaptive immunity in human epilepsies," *Epilepsia*, vol. 58, pp. 57–68, 2017.

[8] A. Vezzani, D. Moneta, C. Richichi et al., "Functional role of inflammatory cytokines and antiinflammatory molecules in seizures and epileptogenesis," *Epilepsia*, vol. 43, Supplement 5, pp. 30–35, 2002.

[9] A. Vezzani, E. Aronica, A. Mazarati, and Q. J. Pittman, "Epilepsy and brain inflammation," *Experimental Neurology*, vol. 244, pp. 11–21, 2013.

[10] B. E. Jones, M. D. Maerz, and J. H. Buckner, "IL-6: a cytokine at the crossroads of autoimmunity," *Current Opinion in Immunology*, vol. 55, pp. 9–14, 2018.

[11] M. Penkowa, A. Molinero, J. Carrasco, and J. Hidalgo, "Interleukin-6 deficiency reduces the brain inflammatory response and increases oxidative stress and neurodegeneration after kainic acid-induced seizures," *Neuroscience*, vol. 102, no. 4, pp. 805–818, 2001.

[12] T. Tanaka, M. Narazaki, and T. Kishimoto, "IL-6 in inflammation, immunity, and disease," *Cold Spring Harbor Perspectives in Biology*, vol. 6, no. 10, pp. 16295-16296, 2014.

[13] M. Pizzi, I. Sarnico, F. Boroni et al., "Prevention of neuron and oligodendrocyte degeneration by interleukin-6 (IL-6) and IL-6 receptor/IL-6 fusion protein in organotypic hippocampal slices," *Molecular and Cellular Neurosciences*, vol. 25, no. 2, pp. 301–311, 2004.

[14] A. R. Burmeister and I. Marriott, "The interleukin-10 family of cytokines and their role in the CNS," *Frontiers in Cellular Neuroscience*, vol. 12, p. 12, 2018.

[15] D. Lobo-Silva, G. M. Carriche, A. G. Castro, S. Roque, and M. Saraiva, "Balancing the immune response in the brain: IL-10 and its regulation," *Journal of Neuroinflammation*, vol. 13, no. 1, pp. 297-298, 2016.

[16] M. Thom, "Review: hippocampal sclerosis in epilepsy: a neuropathology review," *Neuropathology and Applied Neurobiology*, vol. 40, no. 5, pp. 520–543, 2014.

[17] I. Blumcke, R. Spreafico, G. Haaker et al., "Histopathological findings in brain tissue obtained during epilepsy surgery," *The New England Journal of Medicine*, vol. 377, no. 17, pp. 1648–1656, 2017.

[18] B. S. Chang and D. H. Lowenstein, "Epilepsy," *The New England Journal of Medicine*, vol. 349, no. 13, pp. 1257–1266, 2003.

[19] M. Galovic, V. Q. H. van Dooren, T. Postma et al., "Progressive Cortical Thinning in Patients with Focal Epilepsy," *JAMA Neurology*, vol. 76, no. 10, pp. 1230–1239, 2019.

[20] S. Liimatainen, M. Fallah, E. Kharazmi, M. Peltola, and J. Peltola, "Interleukin-6 levels are increased in temporal lobe epilepsy but not in extra-temporal lobe epilepsy," *Journal of Neurology*, vol. 256, no. 5, pp. 796–802, 2009.

[21] T. Alapirtti, K. Lehtimäki, R. Nieminen et al., "The production of IL-6 in acute epileptic seizure: a video-EEG study," *Journal of Neuroimmunology*, vol. 316, pp. 50–55, 2018.

[22] P. Basnyat, M. Pesu, M. Söderqvist et al., "Chronically reduced IL-10 plasma levels are associated with hippocampal sclerosis in temporal lobe epilepsy patients," *BMC Neurology*, vol. 20, no. 1, pp. 1–8, 2020.

[23] P. Basnyat, S. Järvenpää, J. Raitanen, M. Pesu, K. Lehtimäki, and J. Peltola, "A 1-year follow-up study on immunological changes following deep brain stimulation in patients with epilepsy," *Scientific Reports*, vol. 11, no. 1, p. 13765, 2021.

[24] I. E. Scheffer, S. Berkovic, G. Capovilla et al., "ILAE classification of the epilepsies: position paper of the ILAE Commission for Classification and Terminology," *Epilepsia*, vol. 58, no. 4, pp. 512–521, 2017.

[25] P. Kwan and M. J. Brodie, "Definition of refractory epilepsy: defining the indefinable?," *Lancet Neurology*, vol. 9, no. 1, pp. 27–29, 2010.

[26] M. K. M. Alvim, M. E. Morita-Sherman, C. L. Yasuda et al., "Inflammatory and neurotrophic factor plasma levels are related to epilepsy independently of etiology," *Epilepsia*, vol. 62, no. 10, pp. 2385–2394, 2021.

[27] I. F. Uludag, S. Bilgin, Y. Zorlu, G. Tuna, and G. Kirkali, "Interleukin-6, interleukin-1 beta and interleukin-1 receptor antagonist levels in epileptic seizures," *Seizure*, vol. 22, no. 6, pp. 457–461, 2013.

[28] E. E. de Vries, B. van den Munckhof, K. P. J. Braun, A. van Royen-Kerkhof, W. de Jager, and F. E. Jansen, "Inflammatory mediators in human epilepsy: a systematic review and meta-analysis," *Neuroscience and Biobehavioral Reviews*, vol. 63, pp. 177–190, 2016.

[29] Y. Wang, D. Wang, and D. Guo, "Interictal cytokine levels were correlated to seizure severity of epileptic patients: a retrospective study on 1218 epileptic patients," *Journal of Translational Medicine*, vol. 13, no. 1, pp. 1–9, 2015.

[30] W. B. Gallentine, S. Shinnar, D. C. Hesdorffer et al., "Plasma cytokines associated with febrile status epilepticus in children: a potential biomarker for acute hippocampal injury," *Epilepsia*, vol. 58, no. 6, pp. 1102–1111, 2017.

[31] A. Vezzani, J. French, T. Bartfai, and T. Z. Baram, "The role of inflammation in epilepsy," *Nature Reviews Neurology*, vol. 7, no. 1, pp. 31–40, 2011.

[32] S. Aulická, K. Česká, J. Šána et al., "Cytokine-chemokine profiles in the hippocampus of patients with mesial temporal lobe epilepsy and hippocampal sclerosis," *Epilepsy Research*, vol. 180, p. 106858, 2022.

[33] A. Srivastava, A. B. Dixit, D. Paul et al., "Comparative analysis of cytokine/chemokine regulatory networks in patients with hippocampal sclerosis (HS) and focal cortical dysplasia (FCD)," *Scientific Reports*, vol. 7, no. 1, p. 15904, 2017.

[34] A. A. Kan, W. de Jager, M. de Wit et al., "Protein expression profiling of inflammatory mediators in human temporal lobe epilepsy reveals co-activation of multiple chemokines and cytokines," *Journal of Neuroinflammation*, vol. 9, no. 1, pp. 1–22, 2012.

[35] M. W. Aalbers, K. Rijkers, H. J. Majoie et al., "The influence of neuropathology on brain inflammation in human and experimental temporal lobe epilepsy," *Journal of Neuroimmunology*, vol. 271, no. 1-2, pp. 36–42, 2014.

[36] K. J. Meador, D. W. Loring, P. G. Ray, S. W. Helman, B. R. Vazquez, and P. J. Neveu, "Role of cerebral lateralization in control of immune processes in humans," *Annals of Neurology*, vol. 55, no. 6, pp. 840–844, 2004.

[37] S. Bauer, S. Cepok, A. Todorova-Rudolph et al., "Etiology and site of temporal lobe epilepsy influence postictal cytokine release," *Epilepsy Research*, vol. 86, no. 1, pp. 82–88, 2009.

[38] R. C. Sumner, A. Parton, A. V. Nowicky, U. Kishore, and Y. Gidron, "Hemispheric lateralisation and immune function: a systematic review of human research," *Journal of Neuroimmunology*, vol. 240-241, pp. 1–12, 2011.

[39] Y. A. Moshel, H. G. Durkin, and V. E. Amassian, "Lateralized neocortical control of T lymphocyte export from the thymus: I. Increased export after left cortical stimulation in behaviorally active rats, mediated by sympathetic pathways in the upper spinal cord," *Journal of Neuroimmunology*, vol. 158, no. 1-2, pp. 3–13, 2005.

[40] K. A. Lehtimäki, T. Keränen, J. Palmio, and J. Peltola, "Levels of IL-1beta and IL-1ra in cerebrospinal fluid of human patients after single and prolonged seizures," *Neuroimmunomodulation*, vol. 17, no. 1, pp. 19–22, 2009.

[41] K. A. Lehtimäki, T. Keränen, H. Huhtala et al., "Regulation of IL-6 system in cerebrospinal fluid and serum compartments by seizures: the effect of seizure type and duration," *Journal of Neuroimmunology*, vol. 152, no. 1-2, pp. 121–125, 2004.

[42] K. A. Lehtimäki, T. Keränen, J. Palmio et al., "Increased plasma levels of cytokines after seizures in localization-related epilepsy," *Acta Neurologica Scandinavica*, vol. 116, no. 4, pp. 226–230, 2007.

[43] N. Potere, A. Batticciotto, A. Vecchié et al., "The role of IL-6 and IL-6 blockade in COVID-19," *Expert Review of Clinical Immunology*, vol. 17, no. 6, pp. 601–618, 2021.