# Could sP-Selectin and sICAM-1 be potential biomarkers in status epilepticus?

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**Abstract**

**Objective:** To investigate whether soluble P-selectin (sP-selectin) and soluble intercellular adhesion molecule-1 (sICAM-1) might be potential biomarkers that predict the course and prognosis of status epilepticus (SE).

**Patients and Methods:** Fourty-two adult patients with SE between February 2012 and December 2013 were included in the study. Clinical and demographic features of the patients were recorded and surviving patients were followed for 13.6 ± 4.6 months. Serum sICAM-1 and sP-selectin levels were measured during SE or within 24 hours of SE, and compared with 28 subjects in the control group.

**Results:** Levels of serum sP-selectin and sICAM-1 were higher in the SE group compared with the control group (P: 0.04 and P: 0.02, respectively). It was shown that higher levels of serum sICAM-1 correlated with poor outcomes (P: 0.017) and "ROC curve" analysis showed that levels higher than 457 ng/mL predicted poor outcomes with 71% sensitivity and 68% specificity. Levels of serum sP-selectin did not correlate with outcomes. Subgroup analyses revealed levels of serum sICAM-1 were significantly higher in the epilepsia partialis continua (EPC) group compared with the control group (P: 0.012) and levels of serum sP-selectin were not different between subgroups and controls. Levels of serum sP-selectin and sICAM-1 didn't differ between subgroups of SE and different etiologies.

**Conclusion:** Higher levels of serum sICAM-1 may predict poor outcome in SE, as a result sICAM-1 may be used as a biomarker of the prognosis of SE in clinical practice. The production of sICAM-1 may increase particularly in EPC. However, no correlation was found between etiology of SE and serum level of sICAM-1, even in patients with EPC. Serum level of sP-Selectin is not an appropriate biomarker for the prognosis of SE. Serum levels of sICAM-1 and sP-selectin are not appropriate biomarkers of refractory SE.

**Keywords:** status epilepticus, biomarker, sP-selectin, sICAM-1

## Introduction

Biomarkers of epileptogenesis and ictogenesis may exist and in turn, these might be used to predict the onset of epileptic episodes and determine the presence and possible extent of tissue that may predispose to spontaneous episodes. These biomarkers also have the potential to be used to evaluate prognosis once the clinical episode has settled and to create animal models for more cost-effective screening of potential antiepileptogenic and antiseizure drugs and devices, and reduce costs in potential antiepileptogenic and antiseizure clinical studies by enriching the study population with patients at high risk for epilepsy. Biomarkers of epilepsy might be found via imaging modalities, electrophysiologic measurements, and molecules measured in tissue, blood, or cerebrospinal fluid (CSF) (1).

Brain injury that results from status epilepticus (SE) is mainly due to the excitotoxic damage caused directly by seizures (2). Biomarkers of brain injury would be useful in determining critical seizure duration in terms of detecting neuronal injury and defining epilepsy subtypes that may require more aggressive treatment, thus serving as prognostic markers. Several biomarkers have been correlated to epilepsy and brain injury. Brain-derived neurotrophic factor (BDNF), (3) myelin basic protein (MBP), (4) and glial fibrillary acidic protein (GFAP), (4, 5) neurofilament heavy chain protein and heat shock protein (HSP-70) (6) as well as various enzymes (enolase, aldolase, pyruvate kinase, lactate dehydrogenase, creatine phosphokinase), (7) metalloproteases, (8) tau protein, (9) neuron-specific enolase (NSE) (10) and S100B protein (11) in particular, have been investigated.

It has recently been demonstrated that modification of astroglial function, immune/inflammatory reactions, and impairment of blood brain barrier also contribute to brain injury resulting from seizures (12-14).

Cellular adhesion molecules are known to play important roles in inflammatory pathologies (15-17) particularly including the pathogenesis of atherosclerosis (18) and several neurologic diseases such as ischemic stroke, multiple sclerosis, and Alzheimer's disease (19) by mediating the migration of immune cells into the inflamed tissue. Adhesion molecules on endothelial cells and immune cells are termed soluble cellular adhesion molecules when shed into the circulation, and might be determined using an enzyme-linked immunosorbent assay (ELISA) (20).

Animal studies have demonstrated that seizures cause an inflammatory wave, which results in the increased synthesis of adhesion molecules, (12, 21, 22) and increased soluble intercellular adhesion molecule-1 (sICAM-1) synthesis via intraventricular injection of kainic acid (23). Furthermore, administration of monoclonal antibodies aimed at adhesion molecules following pilocarpine-induced seizures has provided a marked reduction in seizures (22). However, few human studies have investigated the relationship between seizures and adhesion molecules (24-27).

The objective of this study was to compare levels of sP-selectin and sICAM-1 in patients with SE and healthy controls to determine whether these could be potential biomarkers that predict the course and prognosis of SE.

## Material and Methods

### 1 Patients and Clinical Data

Fourty-two adult patients who were diagnosed as having SE following referral to the Emergency unit of the Neurology Department or during their hospitalization in other clinics of Istanbul Medical Faculty of Istanbul University between February 2012 and December 2013 were included in the study, and their demographic and clinical features in addition to early and late prognosis were recorded prospectively. At first there were 50 patients, but 8 patients who had cerebrovascular disease were excluded because serum levels of adhesion molecules are known to be affected by atherosclerotic risk factors (28). More patients would be expected to be included in such a study but written informed consents could only be obtained for 50 patients from them or their close relatives when the patients were in SE. The study was approved by the Ethics Committee of Istanbul Medical Faculty of Istanbul University prior to the performance of any study procedures. All patients and volunteering controls were informed about the study outline and examinations, and written informed consent was obtained from all participants or their relatives if they were unconsciousness due to SE.

### 2 Blood Samplings

Blood samples were drawn from all subjects in the study and control groups during or within 24 hours of SE, and centrifuged for 10 minutes at 3000 revolutions per minute to obtain serum. Serum samples were then stored at -80°C until required for analysis. Levels of sP-selectin and sICAM-1 were measured in the serum samples using the commercial ELISA kits (Bender MedSystems, Vienna, Austria) at the Central Biochemistry Laboratory of the Department of Biochemistry of Istanbul Medical Faculty of Istanbul University.

Levels of sP-selectin were measured in 42 patients with SE and 26 controls, and sICAM-1 levels were measured in 40 patients with SE and 28 controls. The discrepancy between the number of patients and control samples was due to a lack of serum samples. The control group consisted of age- and sex-matched healthy volunteers who worked in our hospital or relatives of patients who were hospitalized. Control population did not have any inflammatory or infectious disease, neoplasms, hypertension, diabetes mellitus, chronic hepatic and renal disfunction, cerebrovascular disease which may alter serum levels of soluble adhesion molecules.

### 3 Seizure Types

Generalized convulsive status epilepticus (GCSE) was defined as one seizure or two seizures, without attainment of consciousness in-between, lasting longer than 5 minutes (29). The diagnosis of epilepsia partialis continua (EPC) was established by the observation of a seizure with contractions involving only focal parts of the body and lasting at least 30 minutes; consciousness is often preserved although various degrees of altered consciousness might be observed (30). The diagnosis of non-convulsive SE (NCSE) was established through an electroencephalogram (EEG) (31). Refractory SE (RSE) was defined as SE refractory to treatment with first-line benzodiazepines (BZD) and any of the second-line antiepileptic drugs (AED) including phenytoin (PHT), valproic acid (VPA), levetiracetam (LEV), and phenobarbital (PB) (32).

### 4 Treatment

Patients with GCSE were initially treated with intravenous (i.v.) 10 mg diazepam (Diazem®; 10 mg given over 2-5 min). If seizures continued, another 10 mg diazepam was administered and followed by either one or two of i.v. LEV (Keppra®; 20-60 mg/kg given over 10 min), PHT (Epitoin®, Epanutin®; 15-20 mg/kg given at a rate of 50 mg/min), or VPA (Depakin®; 20-40 mg/kg given over 10 min). Refractory GCSE was treated by anaesthetic doses of barbiturates (Pentothal Sodium® (thiopental); 2-3 mg/kg bolus followed by 3-5 mg/kg/h infusion), midazolam (Dormicum®; 0.2 mg/kg bolus followed by 0.1-0.4 mg/kg/h infusion) or propofol (Propofol®, Pofol®; 3-5 mg/kg bolus followed by 5-10 mg/kg/h infusion); the anaesthetics were titrated against an electroencephalogram burst suppression pattern for at least 24 h. Patients with NCSE and EPC were initially treated in same manner as patients with GCSE. But if they became refractory, further non-anaesthetising i.v. substances such LEV, PHT or VPA were administered instead of anaesthetics. In patients with medical co-morbidities and multiple drugs usage, we prefered LEV instead of PHT and VPA. Brand names of the drugs used in Turkey were given with their doses in brackets.

## 5 Prognosis

Prognosis was determined in two ways: 1) Early poor prognosis was defined as death or neurologic sequelae due to SE within 30 days of SE as in several previous studies, and 2) Late poor prognosis was defined as death or neurologic sequelae due to SE in the late period (at the end of 13.6 ± 4.6 months of follow-up). All other conditions except death and neurologic sequelae were defined as good prognosis.

## 6 Statistical Analysis

The study data was analyzed using the Statistical Package for Social Sciences (SPSS) version 18, and a P value of <0.05 was considered statistically significant. Parametric (independent samples t-test and one-way ANOVA) and non-parametric tests (Mann-Whitney and Kruskal-Wallis tests) were used to compare serum levels of biomarkers in the study and control groups, and to determine their relationship with the prognosis, etiology and refractoriness of SE. Bonferroni and Dunnett were used for post hoc analysis in ANOVA. "ROC curve" was used to determine a cut-off value for sICAM-1 levels to predict poor outcomes.

When power analysis was done with 26 patients and 26 controls, the value was 0.85. We had 42 patients and 28 controls in this study.

## Results

Fourty-two patients were diagnosed as having SE; 19 were males (45.2%) and 23 were females (54.8%) and their ages ranged between 17 and 90 years (mean: 50.5±18.9 years). Classification of patients based upon the SE subtype revealed GCSE in 25 patients (59.5%), EPC in 10 patients (23.8%), and NCSE in 7 patients (16.7%). Eleven patients (26.2%) were diagnosed as having RSE. Early prognosis was good in 31 (73.8%) and poor in 11 (26.2%) patients, and late prognosis was good in 20 (47.6%) and poor in 22 (52.4%) patients. One patient with NCSE and another patient with GCSE developed neurologic sequela directly related with SE. All other patients with poor outcomes died of underlying etiologies and their complications.

Detailed analysis of etiologic factors established that the underlying cause of SE was tumors in 9 patients (21.4%); infectious/metabolic/toxic causes in 10 (23.8%), including 3 patients with encephalitis (7.1%); discontinuation of anti-epileptic drugs in 4 (9.5%); genetic causes in 3 (7.1%); sequelae (operated tumors, head trauma) in 3 (7.1%) patients. The less common etiologic factors were as follows: Multiple sclerosis, anoxia, hippocampal sclerosis, neuronal migration abnormality and cerebral hemorrhage due to leukemia and thrombocytopenia in 1 patient (2.4%) for each. The etiologic factor could not be determined in 8 patients (19%). The demographics, clinical features and etiologies of the patients are presented in Table 1.

Levels of serum sP-selectin and sICAM-1 were compared between the SE and control groups, in subgroups of SE (GCSE, NCSE and EPC) and the control group and only in subgroups of SE. Also levels of these molecules were compared in different etiologies.There were 28 subjects in the control group, which consisted of 12 men (42.8%) and

16 women (57.2%) with a mean age of 43±13.5 years. The control and study groups were sex-matched. The mean age of the control group was lower than the study group without a statistically significant difference (P: 0.075).

Levels of serum sP-selectin and sICAM-1 were higher in the SE group compared with the control group with P values of 0.04 and 0.02, respectively (Table 2). It was shown that higher levels of serum sICAM-1 correlated with late poor prognosis, and "ROC curve" analysis showed that levels higher than 457 ng/ml predicted poor outcome with 71% sensitivity and 68% specificity. The area under the curve was 0.721 and P value was 0.017 (Figure 1). Also, high serum levels of sICAM-1 slightly correlated with early poor prognosis (P: 0.047). There was no correlation between levels of sICAM-1 and refractoriness of SE. No correlation was established between levels of sP-selectin and the prognosis or refractoriness of SE (Table 3).

Subgroup analyses revealed levels of serum sICAM-1 were significantly higher in EPC group compared with the control group (P: 0.012). In subgroup analyses, other comparisons for sICAM-1 (GCSE vs control, and NCSE vs control) and all comparisons for sP-selectin (GCSE vs control, NCSE vs control and EPC vs control) revealed no differences. No correlation was established between levels of sICAM-1 with the prognosis and refractoriness of SE in the EPC group, but levels of sICAM-1 were higher in patients whose SE lasted more than 6 hours (n=5) compared with those whose SE lasted less than 6 hours (n=5) (P: 0.011). Minimum SE duration was 90 minutes and maximum was 2 months in patients with EPC. Serum levels of sP-selectin and sICAM-1 in the SE subgroups and controls are presented in Table 4. Bonferroni and Dunnett tests for post hoc analysis in ANOVA for multiple comparisons of sICAM-1 are shown in table 5.

We could not find relationship between poor outcome in the short and long period and refractoriness (P values were 0.1 and 0.3, respectively). Comparisons of sICAM-1 and sP-selectin according to SE groups (GCSE, NCSE and EPC) revealed no differences. The etiologies of the patients were very heteregoneous in this study and we categorized the etiologies to better distinguish those patients with poor outcome where biomarkers gave some clues. First, we made two groups: 1- Patients with brain tumors (n=9) 2- Others (n=33). There was no difference between groups in terms of sICAM-1 and sP-selectin levels (p: 0.76 and 0.37, respectively) in Mann-Whitney Test. Also there was no difference between patients with brain tumors+poor outcome (n=6) and patients with other etiologies+poor outcome (n=16) in terms of sICAM-1 and sP-selectin levels (p: 0.23 and 1, respectively) in Mann-Whitney Test. Second, we made 5 groups: 1-Brain tumors (n=9), 2-Infections (n=5) (CNS or systemic), 3-Metabolic-toxic causes (n=5), 4- Unknown cause (n=11) (We included patients with genetic etiology to this group), 5- Other causes (n=12) (MS, hipocampal sclerosis, bleeding due to thrombocytopenia, double cortex, sequela of trauma, anoxia and operated epidermoid cyst in brain and AED discontinuation (These 4 patients were using AEDs due to prior meningitis, stroke, lesions that were not clear whether they were tumors or caused by candida and possible

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autoimmune encephalitis)). There was no difference between groups in terms of sICAM-1 and sP-selectin levels (p: 0.63 and 0.92, respectively) in Kruskal-Wallis Analysis. Also there was no difference between patients with brain tumors+poor outcome (n=5), infections+poor outcome (n=4), metabolic-toxic causes+poor outcome (n=4), unknown cause+poor outcome (n=5), other causes+poor outcome (n=3) in terms of sICAM-1 and sP-selectin levels (p: 0.49 and 0.86, respectively) in Kruskal-Wallis Analysis.

It was previously shown that serum levels of adhesion molecules were not age dependent in the 18-65 years age range (28) but there is no data for people aged more than 65 years in the literature.

Therefore, we compared the levels of serum sP-selectin and sICAM-1 between patients aged more than 65 years (n=11) and those aged less than 65 years. There was no statistical important difference between groups for sICAM-1 (P: 0.56) and sP-selectin (P: 0.27).

**Table 1:** Demographics, clinical features and etiologies of the patients

**Demographics**
> Males (n=19, 45.2%)
> Females (n=23, 54.8%)
> Mean age: 50.5± 18.9 years

**SE types**
> GCSE (n=25, 59.5%)
> EPC (n=10, 23.8%)
> NCSE (n=7, 16.7%)

**Refractory SE** (n=11, 26.2%)

**Early prognosis**
> Good (n=31, 73.8%)
> Poor (n=11, 26.2%)

**Late prognosis**
> Good (n=20, 47.6%)
> Poor (n=22, 52.4%)

**Etiologies (n=42)**
> Tumors  (n=9, 21.4%)
> CNS infection  (n=3, 7.1%)
> Systemic infection  (n=2, 4.8%)
> Metabolic-toxic  (n=4, 9.5%)
> Metabolic disorder and infection        (n=1, 2.4%)
> Multiple sclerosis    (n=1, 2.4%)
> Sequelae (operated tumors, head trauma)  (n=3, 7.1%)
> Genetic    (n=3, 7.1%)
> Unknown   (n=8, 19%)
> Anoxia     (n=1, 2.4%)
> Cerebral hemorrhage due to leukemia and thrombocytopenia  (n=1, 2.4%)
> Discontinuation of AED      (n=4, 9.5%)
> Neuronal migration abnormality      (n=1, 2.4%)
> HS  (n=1, 2.4%)

CNS: Central nervous system, AED: Antiepileptic drug, HS: Hippocampal sclerosis, SE: Status epilepticus, GCSE: Generalized convulsive status epilepticus, EPC: Epilepsia partialis continua, NCSE: Non-convulsive status epilepticus

**Table 2:** Levels of serum sP-selectin and sICAM-1 in SE and control groups and P values.

|  | SE | Control | P |
|---|---|---|---|
| **sICAM-1 (ng/ml)** | 479.1 ± 168.9 (N=40) | 392.4 ± 109.1 (N=28) | **0.020** |
| **sP-selectin (ng/ml)** | 163.8 ± 63.8 (N=42) | 133.4 ± 47.7 (N=26) | **0.040** |

**Table 3:** Levels of serum sP-selectin and sICAM-1 in SE group according to early and late prognosis and refractoriness and their P values.

|  | Early good prognosis | Early poor prognosis | p |
|---|---|---|---|
| sICAM-1 (ng/ml) | 448.7 ± 154.5 | 570.6 ± 185.2 | **0.047** |
| sP-selectin (ng/ml) | 158.4 ± 60.8 | 179.2 ± 72.5 | 0.360 |
|  |  |  |  |
|  | **Late good prognosis** | **Late poor prognosis** | **p** |
| sICAM-1 (ng/ml) | 410 ± 139 | 542 ± 172.1 | **0.017** |
| sP-selectin (ng/ml) | 159.6 ± 53.5 | 167.7 ± 73 | 0.685 |
|  |  |  |  |
|  | **Non-refractory SE** | **Refractory SE** | **p** |
| sICAM-1 (ng/ml) | 469.6 ± 160.3 | 507.7 ± 198.9 | 0.544 |
| sP-selectin (ng/ml) | 162.4 ± 66.4 | 167.7 ± 58.6 | 0.816 |

**Table 4:** In the table, median, minimum and maximum values (on the first line); mean and standard deviation values (on the second line) of serum levels of molecules are shown. The superscripts a, b, c and d show the subgroups EPC, JCSE, NCSE and controls respectively with the asterisks notation *: P < 0.05 show the significance level of the multiple comparisons of posthoc tests.

| Molecules | EPC | GCSE | NCSE | Controls | ANOVA |
|---|---|---|---|---|---|
| sICAM-1 (ng/ml) | 521.5 (229-931) 551.7 ± 214.5 [d*] | 433 (217-706) 440.2 ± 147.6 | 487 (362-787) 503.6 ± 145.5 | 374 (217-627) 392.4 ± 109.1 [a*] | P<0.05 |
| sP-selectin (ng/ml) | 171 (70.4-230.1) 160.2 ± 49 | 172.7 (11.7-316.3) 168 ± 69 | 150.4 (87.7-290.1) 154.3 ± 70.8 | 137.2 (42.8-256.8) 133.3 ± 47.7 | P: 0.212 |

**Table 5:** Bonferroni and Dunnett tests were used for post hoc analysis in ANOVA for multiple comparisons of sICAM-1.

**Multiple Comparisons**
Dependent Variable:sICAM-1

|  | (I) Groups | (J) Groups | Mean Difference (I-J) | Std. Error | Sig. | 95% Confidence Interval | |
|---|---|---|---|---|---|---|---|
|  |  |  |  |  |  | Lower Bound | Upper Bound |
| Bonferroni | JCSE | EPC | -111,526 | 54,845 | ,277 | -260,85 | 37,80 |
|  |  | NCSE | -63,398 | 62,501 | 1,000 | -233,57 | 106,78 |
|  |  | Healthy subjects | 47,781 | 40,746 | 1,000 | -63,16 | 158,72 |
|  | EPC | JCSE | 111,526 | 54,845 | ,277 | -37,80 | 260,85 |
|  |  | NCSE | 48,129 | 71,354 | 1,000 | -146,15 | 242,41 |
|  |  | Healthy subjects | 159,307* | 53,340 | ,024 | 14,08 | 304,54 |
|  | NCSE | JCSE | 63,398 | 62,501 | 1,000 | -106,78 | 233,57 |
|  |  | EPC | -48,129 | 71,354 | 1,000 | -242,41 | 146,15 |
|  |  | Healthy subjects | 111,179 | 61,185 | ,443 | -55,41 | 277,77 |
|  | Healthy subjects | JCSE | -47,781 | 40,746 | 1,000 | -158,72 | 63,16 |
|  |  | EPC | -159,307* | 53,340 | ,024 | -304,54 | -14,08 |
|  |  | NCSE | -111,179 | 61,185 | ,443 | -277,77 | 55,41 |
| Dunnett t (2-sided)[a] | JCSE | Healthy subjects | 47,781 | 40,746 | ,546 | -51,45 | 147,01 |
|  | EPC | Healthy subjects | 159,307* | 53,340 | ,012 | 29,40 | 289,21 |
|  | NCSE | Healthy subjects | 111,179 | 61,185 | ,195 | -37,83 | 260,19 |

*. The mean difference is significant at the 0.05 level.
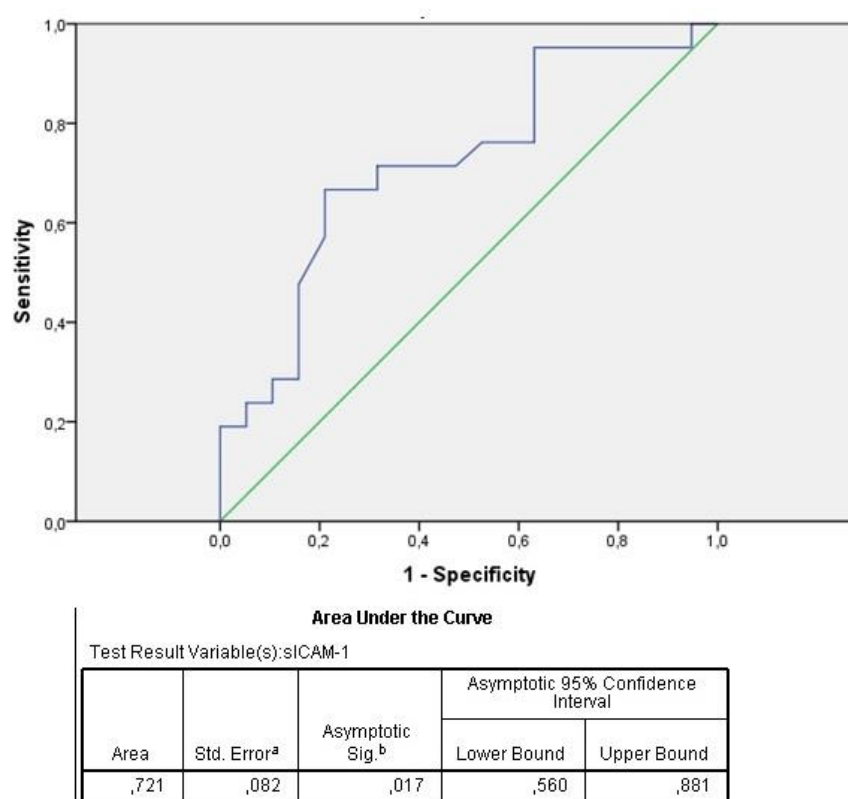a. Dunnett t-tests treat one group as a control, and compare all other groups against it.

**Area Under the Curve**

Test Result Variable(s):sICAM-1

|  |  |  | Asymptotic 95% Confidence Interval | |
| Area | Std. Error[a] | Asymptotic Sig.[b] | Lower Bound | Upper Bound |
| --- | --- | --- | --- | --- |
| ,721 | ,082 | ,017 | ,560 | ,881 |

**Figure 1:** "ROC curve" analysis of serum levels of sICAM-1 predicting poor outcome in status epilepticus. AUC (area under the curve): 0.721, P: 0.017.

## Discussion

Our study determined that serum levels of sICAM-1 were higher in the EPC group compared with the controls and higher serum levels of sICAM-1 predicted poor outcome in SE patients; therefore, we concluded that it could be used as a biomarker of SE in clinical practice. However, serum levels of sICAM-1 and sP-selectin did not change according to either SE types (comparisons in GCSE, NCSE and EPC groups, excluding controls) or etiology.

Refractoriness is expected to correlate with poor outcome. However, in our recently published study, which used univariate analyses, refractory SE was related with poor outcome in the short period (p: 0.013) but unrelated in the long period (p: 0.114) and also lost its significance in the short period when multivariate analyses were performed (33). In this study, we could not find any relationship between poor outcome in the short and long period and refractoriness. Six of our 7 patients with cryptogenic etiology had refractory SE which could cause less worse outcome in patients with refractory SE because patients with unknown etiology may have good outcomes although they have refractory SE. On the other hand, the small size of our patient group could have contributed to this result.

An extensive literature search highlighted the lack of studies that measured serum levels of sICAM-1 and sP-selectin in patients with SE. A human study compared tissue samples obtained from patients with hippocampal sclerosis (HS) during epilepsy surgery with brain tissue samples obtained from patients who died of non-neurologic causes during autopsy. The authors demonstrated CD4 and CD8 positive lymphocytes and diffuse ICAM-1 staining in the hippocampal parenchyma in patients with HS but no lymphocytes and only a weak ICAM-1 staining in the control group (24). A recently published study reported that levels of serum sICAM-1, and serum and CSF sVCAM-1 were higher in patients with drug-resistant epilepsy compared with patients with drug-responsive epilepsy and patients with newly-diagnosed epilepsy, which suggested that these molecules could have an important role in drug-resistant epilepsy (27). Additionally, another study demonstrated that CSF levels of neuronal adhesion molecule-1 (NCAM-1) were lower in patients with drug-resistant epilepsy compared with patients who were drug-responsive, and in both patient groups compared with controls (25). It has also been established that serum levels of anti-inflammatory sICAM-5 were found lower in patients with drug resistant epilepsy (26). The relation between epilepsy and inflammation has been established in a study which indicates that a cascade of inflammatory process are initiated following an epileptogenic event at birth or later in life that contributes to the development of epilepsy and recurrent seizures (34). At a molecular level, seizures induce a series of inflammations in brain endothelial cells leading to an upregulation of IL-1β and its receptor IL-1R1 (35), the complement system (36), and adhesion molecules (P-selectin, E-selectin, ICAM-1 and VCAM-1) (21, 22).

Another result obtained from this study was that serum level of sICAM-1 in particular increases in EPC, which suggests that sICAM-1 may play a role in the pathogenesis of EPC. Although inflammation is known to have an important role in the pathogenesis of the EPC subtype associated with Rasmussen encephalitis (37) which is also related with anti-GluR3 (38) antibodies, several pathologies of the motor strip might also cause EPC and there are no conclusive reports of the relationship between EPC and sICAM-1. EPC is a rare type of focal status epilepticus. Distinct pathologies can cause EPC such as inflammatory disorders 32%, neoplastic disorders 19%, head trauma 16%, vascular disorders 14%, others 3% and unknown 16% which were reported in a review (39). In our study, the following etiologies were observed in the 10 patients with EPC: tumors (n=5), central nervous system infection (n=1), systemic infection and metabolic disorders (n=2), and an etiology that could not be determined (n=2). Although we found increased sICAM-1 levels in the EPC group, we did not find a relationship between levels of sICAM-1 and different etiologies of EPC. This may be due to small number of patients. Also, the production of sICAM-1 may increase as a common pathway, regardless of etiology in EPC. On the other hand, levels of sICAM-1 were higher in patients with EPC whose SE lasted longer than 6 hours in our study which may suggest that production of sICAM-1 increases in time in EPC. No correlation was established between levels of sICAM-1 with the prognosis and refractoriness of SE in the EPC group but the sample size of this group was too small for these analyses and studies with larger number of patients are needed.

Our study had limitations, one was the small sample size, which did not allow for determination of an association between sICAM-1 and sP-selectin serum levels with SE etiology. Another problem was that these serum levels could only be measured once, thus repeat measurements were not taken to determine whether the levels returned to normal after the SE event. Also, measurements were not performed at the most appropriate time periods as 6, 12, or 24 hours after the start of SE. Obtaining these measurements during SE might lead to a false negative result. We did not check for atherosclerotic risk factors in patients, but we mostly excluded patients with atherosclerotic risk factors by excluding those in whom the etiology was cerebrovascular disease. One could also argue that a molecule that is affected by atherosclerotic risk factors is not a good choice for determining the prognosis of SE.

A recently-published study by Trinka et al. (29) proposed 2 time points for SE. The first time point (t1) is the time beyond which the seizure should be regarded as "continuous seizure activity." The second time point (t2) is the time of ongoing seizure activity after which there is a risk of long-term consequences including neuronal injury. In the case of convulsive SE, t1 at 5 min and t2 at 30 min was proposed, and t1 at 10 min and t2 at >60 min were suggested in cases of focal SE with impaired consciousness. Patients who had a generalized convulsive seizure longer than 5 minutes were diagnosed as having GCSE and were included in our study. Of the 44 patients

included, 37 had GCSE longer than 30 min, which is likely to cause neuronal injury according to Trinka et al., but seven of our patients with GCSE had a seizure duration shorter than 30 minutes.

A study performed on rats demonstrated that vascular endothelial cells started ICAM-1 expression 6 hours after intraventricular injection of kainic acid, followed by vascular accumulation and migration of lymphocyte function-associated antigen-1 (LFA-1) positive leukocytes in the hippocampal region (23). Another study on mice revealed that both the production and leukocyte adhesion of ICAM-1, VCAM-1, E-selectin, and P-selectin were increased following pilocarpine-induced seizures or SE. Significant reduction in SE and spontaneous seizures were observed upon treatment of mice with monoclonal antibodies specific to these molecules and granulocyte-specific antibodies (22). A study on humans, as mentioned before, indicates that sVCAM-1 and sICAM-1 could play an important role in the drug-refractory epilepsy (27).

## Conclusion

This is the first study conducted on human subjects to demonstrate the value of serum levels of sICAM-1 and sP-selectin in the prediction of the course and prognosis of status epilepticus (SE). Investigation of these biomarkers on higher number of patients and the determination of the connection between their high levels and outcome of the SE would give us the chance to choose more aggressive antiepileptic drug treatment and to reduce morbidity and mortality in patients with SE. The results of our study and animal studies suggest that the production of sICAM-1 may be increased as a common pathway, regardless of etiology in SE and especially in EPC. However, further studies are required to elucidate the specific role of sICAM-1 and sP-selectin in the pathogenesis of SE, to investigate the relationship between etiology and SE and to consider these molecules as potential targets in SE treatment; because predicting outcome is usually not enough for clinicians and the biomarkers are expected to provide information to develop novel approaches in terms of treatment of the condition.

**Conflict of Interest:** The authors declare no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

**Author's Contributions: MMA, AT, AD, CG:** Research concept and design; data collecting, analysis and interpretation of data. **MMA:** Preparation of article, and Revisions. All authors approved the final version of the manuscript.

**Ethical issues:** All Authors declare, Originality and ethical approval of research. Responsibilities of research, responsibilities against local ethics commission are under the Authors responsibilities.

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