Drug resistant epilepsies: a multicentre case series of steroid therapy

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HIGHLIGHTS

- Drug-resistant epilepsies (DRE) are a challenge for paediatricians.
- The steroid must be considered a therapeutic option in these forms of epilepsy.
- 62.68% of patients presented a reduction in epileptic seizures greater than 50%.
- DREs with structural and immune aetiology respond better than genetic forms.
- The most effective steroid protocol is methylprednisolone 30 mg/kg/day for 3 days.

Drug resistant epilepsies: a multicentre case series of steroid therapy

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Abstract

Purpose: Our study aimed to evaluate the effectiveness of corticosteroids on seizure control in drug-resistant epilepsies (DREs). Our primary goal was to assess the response to steroids for various underlying etiologies, interictal electroencephalographic (EEG) patterns and electroclinical seizure descriptions. Our second goal was to compare steroid responsiveness to different treatment protocols.

Methods: This is a retrospective multicentre cohort study conducted according to the STROBE guidelines (Strengthening the Reporting of Observational Studies in Epidemiology). The following data were collected for each patient: epilepsy etiology, interictal EEG pattern, seizure types and type of steroid treatment protocol administered.

Results: Thirty patients with DRE were included in the study. After 6 months of therapy, 62.7% of patients experienced reduced seizure frequency by 50%, and 6.6% of patients experienced complete seizure cessation. Findings associated with favourable response to steroids included structural/lesional etiology of epilepsy, immune/infectious etiology and focal interictal abnormalities on EEG. Comparing four different steroid treatment protocols, the most effective for seizure control was treatment with methylprednisolone at the dose of 30 mg/kg/day administered for 3 days, leading to greater than 50% seizure reduction at 6 months in 85.7% of patients. Treatment with dexamethasone 6 mg/day for 5 days decreased seizure frequency in 71.4% of patients. Hydrocortisone 10 mg/kg administered for 3 months showed a good response to treatment in 71%.

Conclusions: In our study, two-thirds of patients with DRE experienced a significant seizure reduction following treatment with steroids. We suggest considering steroids as a potential therapeutic option in children with epilepsy not responding to conventional antiseizure medicines (ASM)**.**

KEYWORDS: drug-resistant epilepsies; steroid-therapy; neuroinflammation.

1. Introduction

According to the International League Against Epilepsy (ILAE), drug-resistant epilepsies (DRE) are defined as epilepsies resistant to two or more antiseizure medicines (ASM), appropriately chosen and used whether as monotherapies or in combination [1]. DRE is a challenging condition that affects approximately 10-20% of all children with epilepsy [2-6.]. According to other studies this percentage even rises to 30-40% [7-10].

It is associated with significant morbidity due to the high seizure burden and multiple medications used. Recent studies have shown that neuroinflammation may both precipitate and sustain seizures, implying that inflammation may be involved not only in epileptogenesis but also in the development of the drug-resistant profile. Due to their role in immunomodulation and immunosuppression, steroids have been considered potential candidates in the ongoing search for novel anti-epileptic therapies [11]. The mechanism of action of steroids is thought to be interference with the inflammatory cascade, believed to trigger ongoing seizures [12-15]. With this hypothesis in mind, several different treatment protocols have been used in studies with the goal

of breaking the seizure refractoriness. Evidence supports the use of corticosteroids as treatment for epileptic spasms; however, there are still few studies on their effect on epileptic encephalopathy or drug-resistant epilepsies [16]. The objectives of this study were twofold: first, to evaluate the efficacy of steroids in DRE in children, particularly focusing on aetiology, interictal EEG and seizure types and second, to assess the effectiveness of the steroid treatment protocols used by comparing steroid types.

2. Materials and methods

This retrospective multi-centre cohort study included all DRE patients treated with steroids in paediatric neurology centres of Catania, Padova, Chieti, Troina, Palermo and Rome between 2015-2017. This study conformed to the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) Statement, all points on the checklist have been respected.

2.1 Eligibility criteria

Children with DRE and meeting the following criteria were included:

1) Diagnosis of DRE according to the classification of the ILAE [1].
2) Treatment with steroids as an add-on agent to the other ASM (reason 1).
3) Treatment with at least two ASMs for a minimum of 4 months (reason 3).
4) No change in ASM was found for at least 8 weeks immediately before inclusion in our study and also during treatment and during follow-up (reason 4).

The exclusion criteria were:

1) Patients under 6 months and over 14 years of age (reason 2).
2) Patients diagnosed with any of the following: infantile spasms syndrome, syndrome of electrical status epilepticus during use spike-wave activation in sleep (SWAS), or Landau-Kleffner syndrome (LKS) (reason 5).
3) Patients with a history of malignancy within the past 3 years prior to randomization (reason 6).
4) Patients with moderate to severe hepatic and/or renal impairment (reason 7).
5) Patients with any severe and/or uncontrolled medical conditions at randomization, such as symptomatic congestive heart failure, congenital heart diseases, or any other clinically significant cardiac disease, or significant symptomatic deterioration of lung function (reason 8).
6) Patient with uncontrolled diabetes or with uncontrolled hyperlipidemia (reason 9).
7) Patients with a history of organ transplant (reason 10).

8) Patients receiving any anti-cancer therapies or who have received anti-cancer therapy within 4 weeks of study entry, including chemotherapy, radiation therapy, and antibody-based therapy (reason 11).

9) Patients receiving chronic, systemic treatment with corticosteroids or another immunosuppressive agent at study entry (reason 12).

2.3 Collected Data

All the authors accurately reviewed the medical records of each patient selected on the basis of the inclusion and exclusion criteria (R.F., A.D.C., S.D.M. and V.S. for Catania; M.V. for Padua; A. V. and G.F. for Chieti; E.M. for Troina ; M.M. for Palermo and A.S. for Rome), the data were extrapolated and discussed within the group.

Written informed consent was obtained from the children's parents or legal guardians.

For each individual patient selected on the basis of the inclusion and exclusion criteria, all data were collected by the doctors of each centre (R.F., A.D.C., S.D.M. and V.S. for Catania; M.V. for Padua; A. V. and G.F. for Chieti; E.M. for Troina ; M.M. for Palermo and A.S. for Rome).

The following data were collected for all study participants: gender, epilepsy aetiology, age at epilepsy onset, seizure type, seizure frequency, interictal video-EEG in wakefulness and sleep, age at treatment initiation, antiepileptic drugs tried, steroid type, steroid treatment dose, duration of steroid therapy, the effect of steroid therapy on seizure frequency and EEG (tables 1 and 2).

Each patient was assigned a random number from 1 to 30 to avoid the names' initials and ensure anonymity.

The patients were classified by the authors of the paper (R. F., A.D.C. and R.L.) into groups based on the following data:

1) Aetiology: in "Structural", "Genetic" and "Immune/Infectious", according to ILAE guidelines [17]

2) interictal EEG: "focal", "multifocal" and "generalised";

3) seizure types: "Generalised Tonic Seizures", "Generalised Tonic-Clonic Seizures", "Focal Tonic Seizures", "Focal Clonic Seizures", "Focal Onset Generalised Tonic Seizures", according to the classification of ILAE [18-20];

4) type of steroid protocol administered.

For each of these classes, we analysed four different temporal instants before ("before therapy" denoted T0) and after the end of therapy ("2 weeks" denoted T15, "1 month" denoted T30 and "6 months denoted T180").

For each patient, we scored the number of daily crises to be able to compare the different times examined.

2.4 EEG

The patients underwent an EEG with synchronised video (video-EEG) [21,22].

Three authors (R.F., C.A.D., and S.D.M) independently evaluated each video-EEG that was compared with the EEG of the same patient performed at a different time (T0, T15, T30, T180).

Fleiss' kappa (K) [23] was used to assess agreement among raters in the electroencephalographic interpretation. An online calculator (dfreelon.org) was used to calculate K. Landis and Koch [24] interpreted the K as follows: <0: low agreement, 0.01–0.2: slight agreement, 0.21–0.4: fair agreement, 0.41–0.6: moderate agreement, 0.61–0.8: substantial agreement, and 0.81–1: nearly perfect agreement.

The protocol we used follows the recommendations of the ILAE and the International Federation of Clinical Neurophysiology [25, 26]. We used head caps with prewired electrodes located according to the full 10–20 pad placement system [27].

We used one electrocardiography (ECG) channel and two electromyography (EMG) channels [25, 26]. The electrode impedances were 5-10 kΩ. A camera recorded all the patient's movements [25, 26].

A 20-30-minute awake recording was used as baseline and it was followed for 5-10 minutes by activation procedures (eye opening and closure, hyperventilation, and intermittent photic stimulation) [25, 26, 28, 29]. Then the patients were asked to go to sleep. According to the recommendations of the ILAE and the International Federation of Clinical Neurophysiology [25, 26], patients underwent partial sleep deprivation or the administration of melatonin (1–3 mg administered 30–60 min before EEG recording) if it was not possible to deprive them. Children younger than 6 years shortened their sleep by 1–3 hours or by an amount deemed necessary to fall asleep at the time of the EEG. Children aged 6 to 12 years went to sleep 2 hours later than usual and woke up 2 hours earlier than usual, remaining awake until the EEG. Children older than 12 years went to sleep 2 hours later than usual, but at the latest at 00 a.m. Stay awake from 4 a.m. until the EEG.

2.5 Drug dosage and assessment of response

Each centre administered the steroid protocol validated by its own hospital to its patients. From our retrospective collection, the following steroid therapy protocols emerged:

1. Intravenous methylprednisolone high dose: 30 mg/kg/day for 3 days (Protocol 1, P1), followed by tapering with oral prednisolone 1.2 mg/kg/day for 4 weeks [30]
2. Intravenous methylprednisolone low dose: 15 mg/kg/day for 5 days, followed by tapering with oral prednisolone 1.2 mg/kg/day for 4 weeks (P2) [31]
3. Oral hydrocortisone: 10mg/kg per day for 1 month, 5 mg/kg per day for 1 month, 2.5 mg/kg per day for 1 month, 1 mg/kg per day for 1 month, and 1 mg/kg on alternate days for 2 months. (P3) [32]

4. Intravenous dexamethasone: 6 mg/Kg per day for 5 days, 5 mg/kg per day for 2 days and 2 mg/kg for 2 days (P4) [33]

Follow-up was performed by a paediatric neurologist (R.F., M. S. and C.A.D.) who documented the therapeutic response. The response to steroid treatment was based on seizure diaries filled out by the patients' parents. Seizure diaries included seizure characteristics, frequency, intensity, and duration, similarly to the study performed by Fisher et al. [34].

Every day, the parents report the number of seizures starting four weeks before therapy and stopping six months after the end of therapy. To compute the number of seizures before therapy for each patient, we calculated the mean of the seizures per day in the four weeks preceding therapy. After the therapy was administered, we calculated an average mean, giving the days a higher weight as the deadline approached. For instance, for T30, the day thirty after therapy of the one-month deadline had a higher weight compared to the day two after therapy.

2.6 Statistical analysis

Statistical analysis has been performed using R (v.4.3.0). First, we tested the population distribution using a Shapiro-Wilk test. Successively, Kruskal-Wallis nonparametric one-way analysis of variance has been used to examine the changes in seizure frequency at "before therapy, at 2 weeks, at 1 month and at 6 months" considering several variables. Moreover, the Wilcoxon test has been employed to compare the intragroup differences at the four observation times (T0, T15, T30, and T180). A p value < 0.05 was considered significant.

2.7 Endpoint

The primary endpoint was to quantify the reduction of seizures in relation to steroid therapy in the follow-up period (T0, T15, T30, T180).

We defined "complete responders" as those patients who were seizure-free (complete cessation of seizures), considering the observation time from the day they finished steroid therapy until the 15th day for T15, the 30th day for T30 and the 180th day for T180. The average was calculated by giving the days a greater weight as the deadline approached.

All patients who experienced greater a seizure reduction than 50% were considered partial responders. On the other hand, less than a 50% reduction or no change (0%) or increase in seizures were considered non-responders.

The secondary endpoint focused on factors that predispose to a better or worse response to steroid treatment (based on the type of aetiology, electroclinical presentation and interictal EEG).

The tertiary and final endpoint was to evaluate the response to the four different protocols.

3. Results

Our database search found 59 patients with a diagnosis of DRE, according to the standard international criteria [1]. Applying the inclusion and exclusion criteria, we collected 30 patients (Figure 1).

3.1 Demographic Data

Of the 30 patients included in the study, 16 were males and 14 were females (table 1). The age of seizure onset ranged from 0 months to 118 months (9 years 10 months), with a mean of 32.7 months (approximately 2 years 10 months). The starting age of steroid treatment ranges from 5 years to 11 years and 11 months, with an average of 77.2 months (about 6 years and 4 months) (table 2).

3.2 Response to the treatment in all patients

In all 30 patients receiving steroid therapy, regardless of treatment protocol, a complete response occurred in only one patient (patient number 29) at 15 days and 1 month of therapy and in two patients (patient numbers 14 and 29) at 6 months of therapy (table 2).

Patient number 14 at 2 weeks and 1 month had a 50% reduction in seizures per day.

Overall, we achieved complete response and partial response (> 50% seizure reduction) in 16.6% (5/30) at 15 days and in 30% (9/30) after 1 month of treatment. The greatest reduction occurred in the 6 months following treatment equal to 62.7%. Therefore, compared to the reduction obtained 15 days after the therapy, the reduction at 6 months was 35% more and compared to 30 days the reduction was 29.4% more.

The non-responders, on the other hand, were 83.3% after 15 days of therapy and 70% after 1 month of therapy, which dropped to 36.6% after six months of therapy.

After six months of therapy, among the patients considered non-responders (11 patients) we have: 4 patients (patients numbers 6, 9, 22 and 26) who presented a 50% seizure reduction, 2 patients who did not respond to therapy (patients numbers 8 and 11) and in only one case we had an increase in seizures (patient number 17).

3.3 Response to treatment according to aetiology of seizures

The aetiologies were as follows (table 1):

A. structural abnormalities (50% of cases, 15 patients): hippocampal sclerosis (3 of 15 patients), polymicrogyria (2 of 15 patients), perinatal ischemic infarct (3 of 15 patients), cortical dysplasia (4 of 15 patients), hypothalamic hamartoma (1 of 15 patients), punctate white matter lesions (1 of 15 patients), injury to cerebrocortical-deep nuclear and perirolandic cortex (1 of 15 patients).

B. Genetic mutation (30% of cases, 9 patients): mutation in *KCNQ2* gene, *CDKL5* gene, *SCN2A* gene (2 patients), *MECP2* gene, *SCN1A* gene, *STXBP1* gene, duplication in *GRIN2A* gene, ring chromosome 20 [(r (20)].

C. immune-mediated/infectious (20% of cases, 6 patients): autoimmune encephalitides (5 of 6 patients) and congenital cytomegalovirus infection.

The patients with structural aetiology underwent genetic tests such as array-CGH, clinic exome sequencing and next-generation sequencing (NGS) for cortical malformations, with negative results.

Considering the three groups of patients according to the aetiology and the three follow-up times, we noticed that at 2 weeks most of the patients did not respond to the treatment (reduction of daily seizures less than or equal to 50%), equal to 86% in the structural aetiology group (13 of 15 patients), 77% in the genetic aetiology group (7 of 9 patients), and 83% in the immune/infectious aetiology group. Rates change markedly at 6 months for the first and third groups, 66% of patients with structural aetiology had a partial (60%, or 9 of 15 patients) or complete (patient number 14) response, and all patients (100%, 6 of 6 patients) with immune-mediated/infectious aetiology responded partially to treatment. Instead of the second group, patients with genetic aetiology, only 33% (3 patients out of 9) presented a partial (patient numbers 27 and 28) and complete response (patient number 29). Among patients with genetic aetiology, patient number 17 is the patient who, after 6 months, showed a 50% increase in daily seizures.

To understand why few of the patients with a genetic cause respond to steroids, we analysed the genetic causes for each patient. Patient number 29, who responded completely and immediately to steroids, has r (20).

The two patients with partial responses to treatment instead present duplication in *GRIN2A* gene (patient number 27) and mutation in the *STXBP1* gene (patient number 28).

Patients who did not respond have mutations in the following genes: *KCNQ2, CDKL5, SCN2A, MECP2,* and *SCN1A*. In particular, patient number 17 who presented an increase in seizures after steroid treatment, has a mutation in the *MECP2* gene and patient number 8, who has an unchanged seizure frequency at 6 months, has a mutation in the *SCN2A* gene.

We can immediately notice a reduction in the number of seizures in patients with structural aetiology and with immune/infectious aetiology before therapy and after two weeks of therapy, one month of therapy and six months of therapy ($p<0,05$) (Figure 2). Moreover, in patients with structural aetiology, we can observe a significant reduction also among two weeks and one month and two weeks and six months ($p<0,05$) (Figure 2 a). A significant reduction is also observed between two weeks and six months of therapy and between two weeks and six months and one month and six months of therapy in patients with immune/infectious aetiology ($p<0.05$) (figure 2b).

In patients with genetic aetiology, we observed a reduction between before therapy and after 2 weeks, before therapy and after 1 month, and before therapy and after 6 months ($p<0,05$) (Figure 3).

3.4 Response to treatment according to interictal EEG

According to the type of interictal EEG, we divided the patients into three groups: a) focal interictal EEG (60% of patients, 18 patients out of 30), b) multifocal interictal EEG (23.3%, equal to 7 patients) and c) generalised interictal EEG (16.6%, 5 patients).

We can notice a reduction in the number of seizures in patients with focal epileptic anomalies before therapy and after two weeks of therapy (p=0,00031), one month of therapy (p=0,00031) and six months of therapy (p=0,00032) (Figure 4). The reduction can be notice even while the therapy is given. It is possible to observe a reduction of seizures number between 2 weeks and 6 months of therapy (p=0,0051), and between 1 month and 6 months of therapy (p=0,023) (Figure 4).

Two weeks after treatment, 88% (16 out of 18 patients) of patients with focal interictal EEG had a non-response to treatment, which decreased to 38% (7 out of 18 patients) after 6 months, all the others had a partial response to treatment (61% after 6 months).

On the other hand, no statistically significant reduction in the number of seizures is evident in the other interictal EEGs ("multifocal" and "generalised").

In the group of patients with multifocal interictal EEG, 71% (5 out of 7 patients) had a non-response to treatment at 2 weeks, while 43% (3 out of 7 patients) were classified as non-responders at 6 months.

Among patients with generalised interictal EEG, patient number 29 was the one who presented a complete response (equal to 100%) from the first two weeks of treatment, while patient number 14 became a complete responder at 6 months of therapy. At 2 weeks of therapy, 80% of the patients in this group were non-responders (4 out of 5 patients) while at 6 months this percentage halved (40%, 2 out of 5 patients).

3.5 Response to treatment according to seizures type

According to the ILAE classification [8-10], we grouped our patients according to electro-clinical presentation: 1) generalised tonic seizures (3 patients), 2) generalised tonic-clonic seizures (6 patients), 3) focal tonic seizures (9 patients), 4) focal clonic seizures (4 patients), 5) focal onset to generalised tonic clonic seizures (8 patients).

We noted that before steroid therapy, children with focal clonic seizures (13,3% of cases) had more crises than those with focal tonic seizures (30% of cases) (p=0.02) and those with generalised tonic-clonic seizures (20% of cases) (p=0.023) in a significant manner; and children with generalised tonic seizures had more crises than those with focal tonic seizures (p=0.091) (Figure 5).

The only significant reduction occurred for patients with focal tonic seizures, who at 1 month had fewer seizures than patients with focal clonic seizures (p=0,036) (figure 6). However, this difference is not maintained at 6 months. The groups are very heterogeneous and some even consist of a few patients.

3.6 Response to different steroid protocol therapy

In this retrospective study, four different steroid therapy protocols were conducted according to the guidelines adopted by the administering centre's hospital. By grouping the patients into these four groups, we have:

1. seven patients in group P1 (IV methylprednisolone high dose),
2. nine patients in group P2 (IV methylprednisolone low dose),
3. seven patients in group P3,
4. seven patients in group P4.

The greatest reduction percentages are for the protocol with methylprednisolone 30 mg/kg/day for 3 days.

From all patients (7 patients) who were non-responders after 2 weeks of therapy with methylprednisolone 30 mg/kg/did, after 6 months of therapy, 85.7% of patients presented a partial response (5 patients out of 7) and a complete response (patient number 14).

For the group with dexamethasone as early as two weeks after therapy, the percentage of patients with partial (patient number 29) and/or complete responses (3 patients out of 7) was 57%, which increased to 71.4% after 6 months of therapy (4 patients were partial responders and patient number 29 were complete responders).

The other two protocols lead to a reduction in the number of seizures throughout the follow-up. After 6 months of therapy, the percentage of patients who presented a reduction in the number of seizures greater than 50%: 33% for the group that received methylprednisolone 15mg/kg/day for 5 days, and 71% for the group receiving hydrocortisone therapy.

We also compared the single protocol with the different aetiologies, but unfortunately the patients for each group are too few (2-4 patients per group) and therefore all the data are not significant.

Discussion:

In this retrospective study, we analysed the efficacy of steroid treatment for children with DRE, the most complex form of epilepsy to manage, as they present a non-response or only a slight reduction to multiple therapies with different ADEs. We included 30 patients with DRE, according to the international definition of the disorder [1]. Our data showed a statistically significant seizure reduction after 2 weeks and 1 month of therapy and a greater reduction after 6 months of treatment. In particular, after 6 months of therapy, 62.7% of patients (19 out of 30 patients) presented a reduction in seizures greater than 50%, 6.6% (2 out of 30 patients) responded completely to therapy without presenting seizures.

Our data are confirmed by a 2023 systematic review by Becker LL and Kaindl AM [16]. They found 16 papers in which a steroid and/or ACTH are used in DRE. Excluding the papers in which ACTH therapy is performed (5 papers), 66% of patients (equal to 286 out of 431 patients) presented a seizure reduction greater than 50%, of which freedom from epileptic seizures was achieved in 67 patients (equal to 15.5% of all patients).

The use of steroids is based on experimental and clinical results that support the crucial role of inflammatory processes in epileptogenesis [35-40]. Inflammation is both a cause (inflammation that is chronic or lasts long enough to cause tissue damage or dysfunction) and a consequence of seizures. Recent studies have suggested that neuroinflammation may both precipitate and sustain ongoing seizures, leading to the theory that the neuroinflammatory process may be involved not only in epileptogenesis but also in the development of the drug-resistant profile [11].

As demonstrated by experimental evidence on rodents, epileptic seizures increase the release of inflammatory mediators in the brain regions involved in the generation and propagation of epileptic activity; conversely, a site of inflammation increases neuronal excitability [41-52].

Indeed, in both animal (rodent) and human (DRE patients with cerebrospinal fluid or brain parenchyma after surgical resection) studies, several proinflammatory agents such as interleukin (IL)-1β, IL-6, TNF, prostaglandin E2 and a highly mobile complement system group 1 (HMGB1) were identified [41-47, 53.-58]. Recent evidence demonstrates that IL-1β activation of the neuronal IL-1 receptor induces activation via N-methyl-D-aspartate (NMDA) receptor phosphorylation, inhibits reuptake, and enhances glutamate release [59-65]. The increase in glutamatergic transmission is the cause of the greater neuronal excitability.

As a result, immunomodulatory therapies have been considered as potential candidates in the ongoing search for novel anti-epileptic drugs [11].

It has been shown that steroids, in addition to interfering in the cerebral inflammatory cascade, have a direct cerebral action. In particular, some steroids (such as progesterone, estradiol, testosterone, glucocorticoid, ganaloxone, androsterone, pregnenolone, etc.) can bind to neurotransmitter receptors and modulate the neurotransmission signal; for this reason, they are called "neurosteroids" or "neuroactive steroids"

[66, 67]. Steroids can bind to synaptic receptors and voltage-gated ion channels, especially GABAA, NMDA, AMPA, etc. [67]. At the cellular level, in addition to the effect on postsynaptic receptors, most steroids have modulatory effects on the release of multiple neurotransmitters (such as glutamate, GABA, acetylcholine, norepinephrine, dopamine, and 5-HT) [68].

Many of these effects occur in brain regions involved in learning and memory, emotions, motivation, motor skills and cognition [68, 69].

It has therefore been well studied in the literature why the steroid acts on epilepsy, but there is a lack of data demonstrating why there is a heterogeneous response in DRE forms.

In our paper, we have studied all patients with DRE to understand what factors predispose to a better or worse response to steroid treatment.

We evaluated the response based on the different aetiologies; in particular, the patients who presented a statistically significant reduction were those with an immune/infectious aetiology (equal to 100% after 6 months) and a structural aetiology (equal to 66%).

However, most patients with genetic aetiology did not show a reduction in the number of daily seizures after steroid treatment. In particular, 77% after 2 weeks and 66% after 6 months of therapy presented a reduction in epileptic seizures of less than or equal to 50%. A patient with genetic aetiology (patient number 17) after 6 months of therapy had a 50% increase in seizures per day compared to before therapy.

In all forms of DRE with an immune/infectious aetiology, there is activation of both the innate and adaptive immune systems, resulting in an inflammatory response primarily involving resident brain cells such as glia and neurons, which is the cause of seizures resistant to ASM [39, 70-72].

Instead of the forms with structural aetiology, it has been demonstrated that the brain tissue presents a strong inflammatory response around the structural alteration of the brain tissue. For example, in the cortical tubers of tuberous sclerosis, the presence of macrophages and alterations in the expression of TNF-α, NFKB and cell adhesion molecules were found [73, 74], the same for cortical dysplasia [54, 75, 76] and other structural alterations [77, 78].

Among the genetic forms, only three patients responded to treatment and present: duplication grin2a (patient number 27), mutation in the stxbp1 gene (patient number 28) and r (20) syndrome. Among these, only one patient presented absence of seizures already after 15 days of therapy, this patient presents r (20). Kishore VK et al. report the case of a male patient with r (20) being treated with sodium valproate and lamotrigine at the optimum weight-based dosage (the most effective anti-seizure medicines in r(20), but he did not have any benefit from these drugs [79]. He was administered 30 mg/kg of intravenous methylprednisolone, five consecutive days a month for nine months. After the first five doses of intravenous steroids there was a

drastic reduction in frequency of seizures from 20–40 times per day to 2–3 times per week, which were brief and nocturnal events.

Most genetic forms (6 out of 9 patients) did not respond to treatment. Patient 17 presented a worsening of seizure frequency after treatment; he had a mutation in the *MECP2* gene. No case of a patient with these mutations who has been treated with steroids is described in the literature, so we cannot compare our data.

Patient number 8 presents a partial response after 1 month of therapy and then has a reduction of less than 50% at 6 months and is therefore a non-responder. In the literature, Müller A et al. report a responder rate of 19% after 3 months of treatment with steroids, which drops to zero after 6 and 12 months of treatment [80].

In our paper, we have also noticed a reduction in the number of seizures in patients with focal epileptic abnormalities, which is not present in cases with other interictal ("multifocal" and "generalised") EEGs. This is in keeping with previous studies that showed a good response to steroid treatment in children with focal seizures [11; 81].

No statistically significant differences were detected based on electroclinical presentation. In the literature, no paper studies the response to treatment based on the interictal EEG tracing or on the electroclinical presentation.

The last aim of this study was to evaluate the efficacy of different steroid treatment protocols for seizure control in children with drug-resistant epilepsy. Of the four therapeutic protocols used in our study, the protocol with the best efficacy was methylprednisolone 30 mg/kg/day for 3 days (P1) with a greater than 50% reduction in epileptic seizures at 6 months of treatment of 85.7%, followed by dexamethasone 6 mg/day for 5 days (P4) with a percentage of 71,4% and hydrocortisone 10 mg/kg for 3 months (P3) with a 71% response to treatment.

Unfortunately, the protocols used in the literature are very heterogeneous, including the dosage, the duration of the treatment and the period of observation. In the retrospective study by Pera MC et al. , out of eleven children suffering from epileptic encephalopathy who were administered methylprednisolone 15–30 mg/kg/day for three consecutive days (once a month for four months), eight patients (72.7%) responded to the cycle of therapy, showing a reduction of at least 50% in seizure frequency [82].

Analysing our data, we noticed a different response immediately for dexamethasone. The response to methylprednisolone 30 mg/kg/day and hydrocortisone is completely negative after two weeks of therapy and after 1 month, while for dexamethasone after 2 weeks and 1 month of therapy, 57% of patients present a partial response.

There are several limitations to our study. It is a retrospective study with all the associated limitations [83]. In our study, only a few patients were collected; these are reduced even more if divided by aetiology, interictal EEG, electroclinical presentation, and administration protocol. An important limitation of this study is related to the precise quantification of daily seizures. Unfortunately, we relied on the diary filled out every day by the parents with the attached limits [34].

Conclusions

We believe that our retrospective study strengthens the hypothesis of the efficacy of steroids in the treatment of medically drug-resistant epilepsy.

Our study is the only one that divides patients with DRE based on aetiology, interictal EEG, and electroclinical presentation. It provides evidence to support the efficacy of steroids in immune-mediated/infectious structural forms and in DRE with focal interictal EEG.

There is currently no standard protocol for children with DRE. Our study introduced four protocols used in the main Italian paediatric neurological centres and demonstrates how some steroids give a greater response.

We can conclude that the steroid offers a good response rate in DRE; therefore, in paediatrics, it must be considered as a treatment option in those forms that do not respond to the countless ASM. Larger, prospective randomised clinical trials are needed to establish the most suitable therapeutic protocol for drug-resistant forms of epilepsy. Many more studies are needed on the role of steroids in genetic forms, especially in specific chromosomal and genetic alterations. Larger studies should also evaluate the short- and long-term effects of steroids on behaviour, memory, and development.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Institutional Review Board.

HUMAN AND ANIMAL RIGHTS

All methods were performed in accordance with the ethical standards of the Declaration of Helsinki.

CONSENT FOR PUBLICATION

Each parent has expressed his consent to participate in the study and the publication of the data in an anonymous form.

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AVAILABILITY OF DATA AND MATERIALS

The datasets used and analyzed during the current study are available from the corresponding author on reasonable request.

Conflict of Interest

The authors declare that they have no competing interests.

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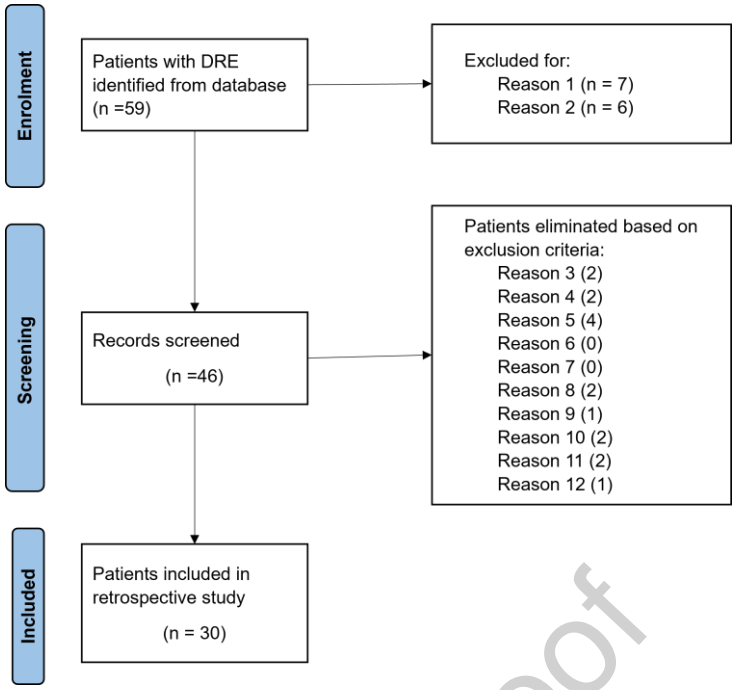

Figure 1: Chart illustrating the flow of participants through the selection criteria.

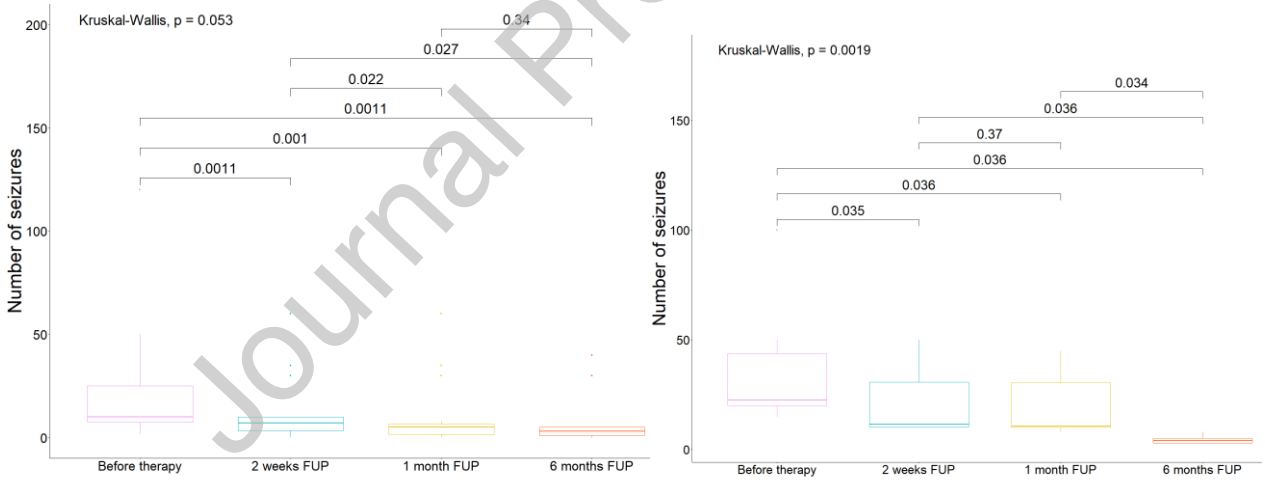

Figure 2: Number of seizures at several temporal instant for patients with structural aetiology **(on the left)** and patients with Immune/Infectious aetiology **(on the right)**.

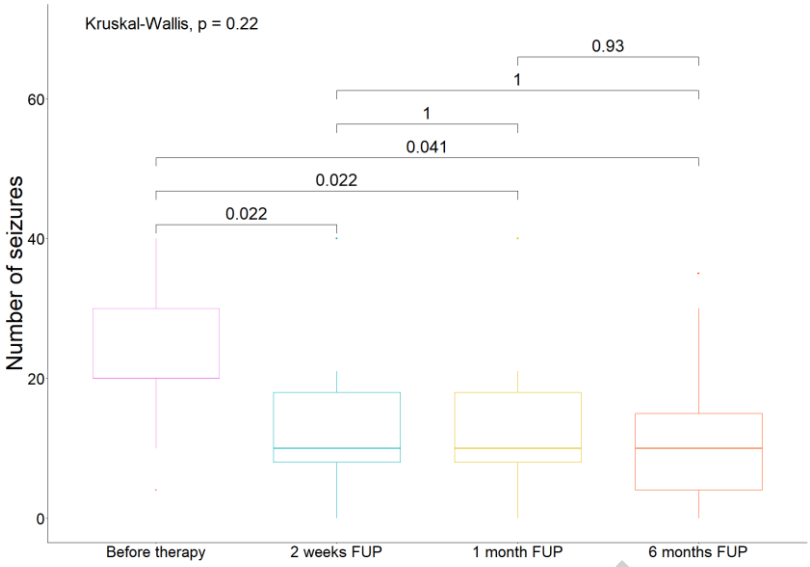

Figure 3: Number of seizures at several temporal instant for patients with Genetic aetiology.

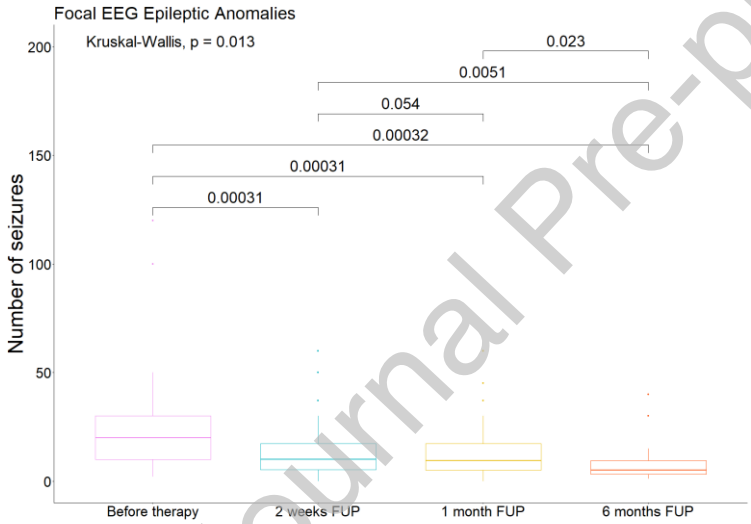

Figure 4: Number of seizures at several temporal instant for patients with Focal Epileptic Anomalies

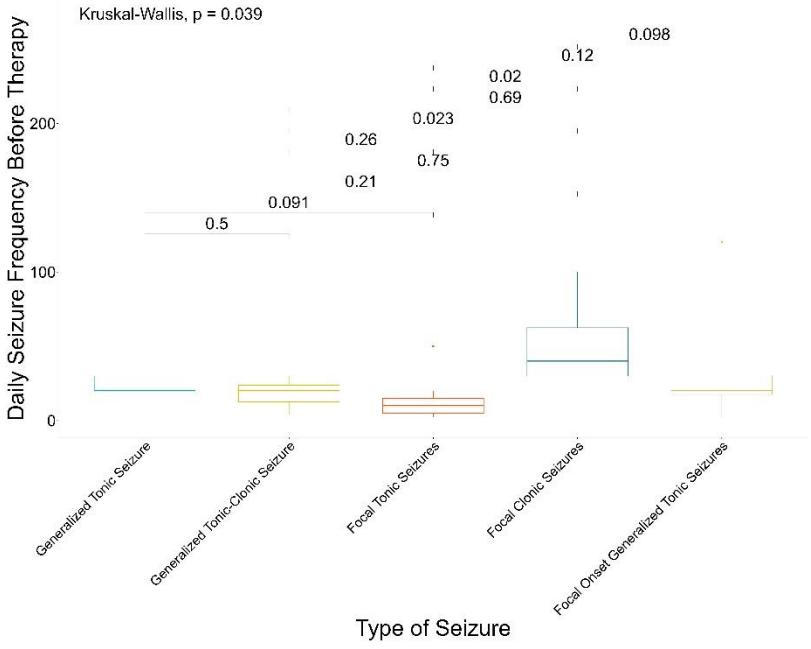

Figure 5: Number of seizures before therapy for different seizure types

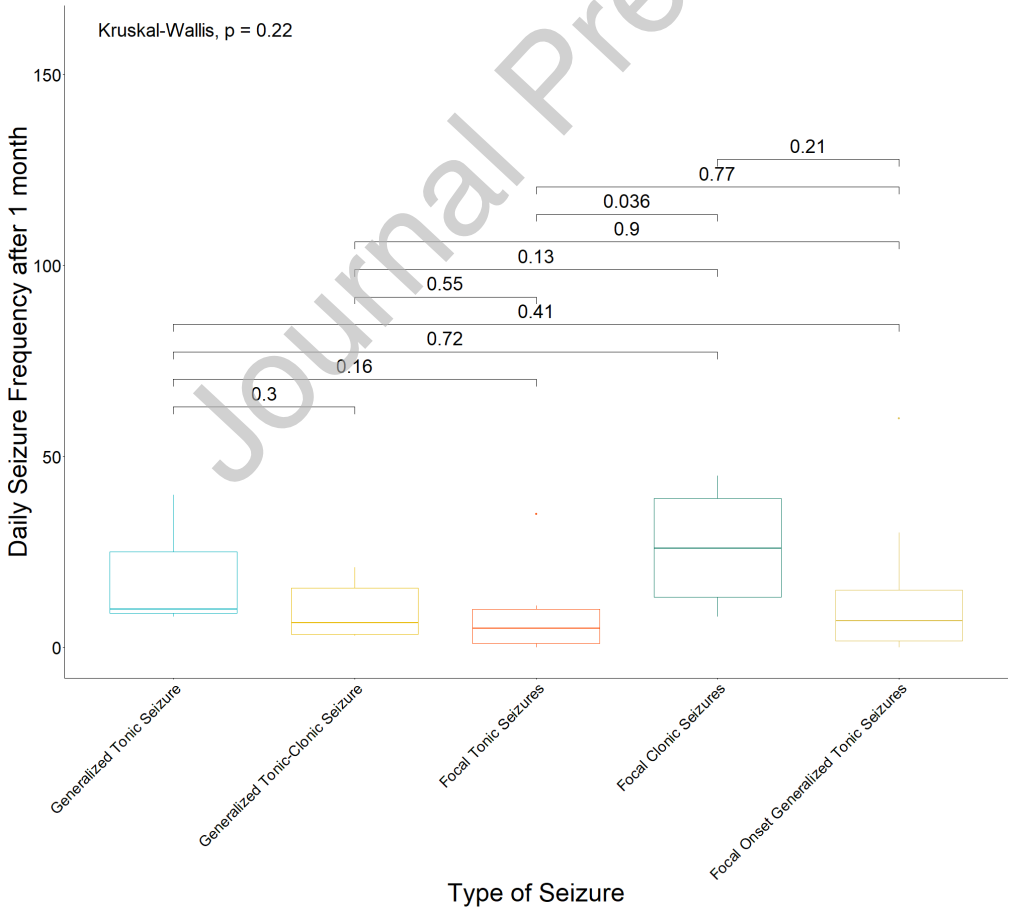

Figure 6: Response to treatment after 1 month for different seizure types

Patients	Gender	Aetiology*		Electro-clinical presentation # [19,20]	Epilepsy syndrome [18]	Age at onset (months)	Frequency (daily)	Interictal - EEG$^{\varphi}$
		According to ILAE classification [17]	Neuroimaging or genetic mutation or infective or immune aetiology					
1	M	A	Hippocampal sclerosis	1	MTLE-HS	90	20	b
2	M	B	*KCNQ2*	1	KCNQ2-DEE	4	40	b
3	F	B	*CDKL5*	5	*CDKL5*-DEE	3	20	a
4	M	A	Polymicrogyria	3	SHE	80	50	b
5	F	C	Autoimmune encephalitides	2	EE	20	25	b
6	M	A	Perinatal ischemic infarct	3	LGS	3	10	a
7	F	C	Autoimmune encephalitides	3	EE	36	15	a
8	F	B	*SCN2A*	2	DEE	8	30	c
9	F	A	Cortical dysplasia	3	LGS	48	10	a
10	M	A	Perinatal ischemic infarct	5	LGS	0	120	a
11	M	A	Hypothalamic hamartoma	5	GS-HH	3	30	a
12	F	C	Congenital cytomegalovirus infection	4	DEE	0	50	a

13	M	A	Punctate white matter lesions	2	LGS	10	10	c
14	M	A	Cortical dysplasia	5	LGS	12	2	c
15	M	A	Polymicrogyria	5	LGS	36	10	a
16	F	C	Autoimmune encephalitides	5	EE	72	20	a
17	M	B	*MECP2*	3	DEE	1	10	b
18	M	A	Cortical dysplasia	3	SHE	52	20	b
19	F	A	Hippocampal sclerosis	5	MTLE-HS	48	30	b
20	M	A	Injury to cerebrocortical -deep nuclear and perirolandic cortex	3	LGS	7	5	a
21	F	A	Cortical dysplasia	3	Focal epilepsy syndrom e with structura l etiology	96	4	a
22	F	A	Hippocampal sclerosis	3	MTLE-HS	12	2	a
23	M	C	Autoimmune encephalitides	4	Focal epilepsy syndrom	118	100	a
24	F	C	Autoimmune encephalitides	1	Focal epilepsy syndrom	24	20	a
25	M	B	*SCN1A*	2	DS	96	4	c
26	F	B	*SCN2A*	2	DEE	3	20	a
27	M	B	Duplication *Grin2A*	4	DEE	72	30	a
28	F	B	*STXBP1*	4	DS	18	30	a

29	M	B	Ring chromosome 20	5	DEE	2	20	c
30	F	A	Perinatal ischemic infarct	2	LGS	8	20	a

TABLE 1: analysed data in all 30 patients.

* Aetiology: A) structural, B) genetic, C) immune/infectious according to ILAE classification[22]

Electro-clinical presentation: 1) generalised tonic seizures, 2) generalised tonic-clonic seizures, 3) focal tonic seizures, 4) focal clonic seizures, 5) focal onset to generalised tonic clonic seizures

ç Interictal EEG a) focal, b) multifocal, c) generalised

M= male, F=female, y= years; m= months;

DEE: developmental and epileptic encephalopathies.

DS= Dravet syndrome

GS-HH= Gelastic seizures with hypothalamic hamartoma

LGS= Lennox–Gastaut syndrome

MTLE-HS= Mesial temporal lobe epilepsy with hippocampal sclerosis

SHE= sleep-related hypermotor (hyperkinetic) epilepsy

Patients	Previous AEDs	Age at initiation of steroid treatment (years and months)	Steroid-protocol[§]	Follow-up Number of seizure/day / Percentage reduction compared to the pre-therapy phase (%)		
				at 2 weeks	at 1 months	at 6 months
1	LEV, VPA, CBZ	10 y and 6 m	P1	10 50%	8 60%	3 75%
2	VPA, LEV, CBZ	5 y and 3 m	P2	40 0%	40 0%	35 12%
3	VPA, LEV, CBZ	5 y and 4 m	P2	10 50%	9 55%	15 25%
4	CBZ, LEV, VPA	5 y and 7 m	P2	35 30%	35 30%	40 20%
5	PHT, VPA, LEV	6 y and 3 m	P2	12 52%	8 68%	3 88%
6	CBZ, LEV	5 y and 5 m	P2	6 40%	5 50%	5 50%
7	CBZ, LEV	6 y	P2	11 26%	11 26%	3 80%
8	VPA, LEV, ETS	6 y	P2	21 30%	21 30%	30 0%
9	CBZ, LEV	6 y and 8 m	P2	5 50%	5 50%	5 50%
10	VGB, RFN, VPA BDZ, PHT	5 y	P3	60 50%	60 50%	40 66%
11	CBZ, LEV, PB, CLB	5 y and 5 m	P3	30 0%	30 0%	30 0%
12	CBZ, VGB, CLB, TPM	5 y and 1 m	P1	37 26%	37 26%	3 94%

13	VPA,PHT	5 y	P1	7 30%	5 50%	3 70%
14	VPA, TPM	5 y	P1	1 50%	1 50%	0 100%
15	VPA, LEV	6 y	P1	5 50%	5 50%	4 60%
16	LEV, CBZ, VPA	8 y and 2 m	P1	10 50%	10 50%	5 95%
17	PB, LEV	5 y and 5 m	P3	10 0%	10 0%	15 -50% (increase)
18	VPA, ETS, CLB	7 y	P3	10 50%	5 50%	1 95%
19	CBZ, VPA, LEV	7 y and 2 m	P4	9 70%	2 93%	2 93%
20	LEV, VPA	5 y and 4 m	P4	0 100%	0 100%	1 80%
21	LCM, PER	10 y and 4 m	P3	2 50%	1 75%	1 75%
22	VPA, LTG	5 y and 1 m	P1	1 50%	1 50%	1 50%
23	OXC, NZ, VPA	11y and 11 m	P2	50 50%	45 55%	8 92%
24	ETS, LEV, VPA,CZP	5 y	P4	10 50%	10 50%	5 75%
25	VPA, ETS, PER	10 y	P4	3 25%	3 25%	3 25%
26	RFM, VPA, CZP	5 y and 10 m	P4	18 10%	18 10%	10 50%
27	TPM, LCM	8 y and 2 m	P4	8 73%	8 73%	4 87%
28	OXC, CZP	5 y and 3 m	P3	15	15	10

				50%	50%	67%
29	CZP, LEV (previously CBZ, VPA, NZ)	5 y	P4	0 100%	0 100%	0 100%
30	TPM, VPA	5 y	P3	10 50%	3 85%	5 75%

Table 2: the treatment in all 30 patients

§ Steroid-protocol:

P1) Intravenous methylprednisolone 30 mg/Kg/day for 3 days (Protocol1, P1), followed by tapering with oral prednisolone 1.2 mg/Kg/day for 4 weeks,

P2) Methylprednisolone 15 mg/Kg/day for 5 days, followed by oral prednisolone 1.2 mg/Kg/day for 4 weeks

P3) Oral hydrocortisone 10mg/kg per day for 1 months, 5 mg/kg per day for 1 month, 2.5 mg/kg per day for 1 month, 1 mg/kg per day for 1 month, and 1 mg/kg on alternate days for 2 months.

P4) Intravenous dexamethasone 6 mg/Kg per day for 5 days, 5 mg/kg per day for 2 days and 2 mg/kg for 2 days .

BDZ= benzodiazepine; CBZ = carbamazepine; CLB= clobazam; ETS= ethosuximide; LCM= lacosamide; LEV= levetiracetam; LTG = lamotrigine; NZ= nitrazepam; OXC= oxcarbazepine; PB = phenobarbital; PER= perampanel; PHT = phenytoin; RFM=rufinamide; TPM = topiramate; VPA = valproic acid; ZP = clonazepam.

Conflict of Interest

The authors declare that they have no competing interests.