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Immunological markers of drug resistant epilepsy and its response to immunomodulatory therapy with ACTH in children

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Abstract

Introduction: *Drug-resistant epilepsy in infancy and childhood is a devastating condition, frequently associated with neuropsychiatric comorbidities. West syndrome is one of the most severe epilepsy syndromes. Adrenocorticotropic hormone (ACTH) treatment is recommended in such cases, but its mechanism of action is still unknown. We prospectively observed levels of selected cytokines in order to identify biomarkers of response to ACTH and the potential mechanism of its antiseizure effect.*
Material and methods: *Fifty-three infants and young children with pharmacoresistant epilepsy receiving $ACTH_{1-24}$ were included. There were 2 control groups – children with epilepsy responding to the first medication and children with no history of epilepsy. Blood concentrations of IL-1β, IL-1Ra, IL-6, IL-8, IL-10, TNF-α, IFN-γ, MCP-1 and MIP-1α were analyzed at three time points: T0 (before ACTH), T1 (after intensive ACTH treatment) and at T2 (at the end of ACTH withdrawal). The results were correlated with the response to treatment, dose of ACTH and concomitant medications.*
Results: *We found statistically significantly higher concentrations of IL-1, IL-8 and MIP-1α at baseline (T0) in the study group compared to the control groups. ACTH significantly lowered levels of IL-6, IFN-γ and MCP from time T0 to T1. This effect was short lasting and no significant changes in cytokine levels were found between T2 and T0. We did not find any differences in immunological markers between the responders and non-responders to ACTH. Our research did not allow us to identify any reliable immunological marker of response to ACTH treatment. We did not observe a positive effect of higher ACTH doses on the response rate in the patients. Our study showed significantly lower concentrations of IL-10 and IFN in the group of patients receiving levetiracetam. The concentration of TNF was higher and the concentration of MIP was lower in the group receiving valproic acid.*
Conclusions: *Our study indicates that increased levels of IL-1, IL-8 and MIP-1α are associated with drug-resistant epilepsy in infants and young children and might be considered immunological markers. IL-6, IFN-γ and MCP-1 take part in the effect of ACTH. Immunological mechanisms seem also to be involved in the mechanism of action of classical antiseizure drugs.*

Key words: *cytokine, ACTH, epilepsy.*

Introduction

Epilepsy is the most common neurological disorder, affecting around 1% of the general population [6,10]. Despite the progress in the understanding of epilepsy mechanisms and the increasing number of approved antiseizure medications, still about one third of the patients suffer from seizures that are resistant to treatment [26,33]. In the young pediatric population (0-24 months of age) the risk of developing pharmacoresistant epilepsy is even higher [34].

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It has been over 70 years since adrenocorticotropic hormone (ACTH) was introduced for the treatment of seizures in children [24].

At present, ACTH is strongly recommended for the treatment of infantile spasms, the epileptic syndrome that is known for its severity and drug resistance [16,18]. It is also recommended in other epilepsy syndromes [9,29]. Despite such a long history of clinical application, the exact antiepileptic mechanism of action of ACTH remains unknown [5,20,23].

There are two main ACTH preparations applied in seizure management – one is the $ACTH_{1-39}$ natural preparation from the pig pituitary gland (ACTHARGEL), used mostly in the USA, and the other is a synthetic, long acting $ACTH_{1-24}$ analogue (tetracosactide, tetracosactrin, cosyntropin), used in European countries and in Japan.

There is no international consensus regarding the ACTH treatment regimen [2]. Even in Europe each country or even each center has its own treatment schedule [16,22,31]. In Japan there is a tendency to use extremely low doses [11].

Immunological mechanisms in epilepsy have become a hot topic in neurology in recent years. Increased synthesis of inflammatory cytokines' and chemokines' mRNA was detected in animal models of epilepsy as well as in the epileptogenic foci obtained from patients operated on for drug-resistant epilepsy [1,30]. It was shown that children with febrile seizures have increased blood levels of the pro-inflammatory interleukin (IL)-1β and tumor necrosis factor α (TNF-α, formerly known as cachexin) in the cerebrospinal fluid up to 12 hours from the onset of febrile seizures [17,28]. It has already been proven that TNF-α is an important disruptor of the blood-brain barrier (BBB), which is one of its pro-convulsant effects [7]. In addition, TNF-α together with IL-1β modulates neurotransmission through AMPA-type glutamatergic receptors (alpha-amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptors), which may cause both pro- and anticonvulsant effects in this mechanism [3]. Another study reported significantly increased levels of IL-1Ra and IL-6 in the blood of children with febrile seizures compared to children with fever without seizures [32]. In the observation of a homogeneous group of patients with drug-resistant temporal lobe epilepsy undergoing surgical treatment in the form of resection of the temporal lobe pole with amygdalohippocampectomy, a significant reduction in the concentrations of IL-1β, TNF-α and macrophage inflammatory protein 1α (MIP-1α/CCL3 chemokine) was observed after surgery in comparison to the value before resection [21]. Increased levels of pro-inflammatory cytokines such as IL-2, TNF-α and interferon α (IFN-α) have been found in the blood of children diagnosed with West's syndrome prior to treatment [14]. In patients with Rasmussen syndrome, T cell infiltration, especially cytotoxic T $CD8^+$ cells, and increased presence of chemotactic cytokines such as CCL5, CXCL10, CCL22, CCL23, CXCL9 and interferon γ (IFN-γ) were observed in the areas of the brain affected by inflammation [19]. Up to now there are several experimental and recognized immunological treatments of seizures. They include corticosteroids, immunoglobulins, immunosuppressive agents such as azathioprine, and monoclonal antibodies such as rituximab, each having a specific mechanism of action [4].

The goal of our study was to observe the concentrations of selected immunological parameters in patients with pharmacoresistant seizures and to analyze their behavior during treatment with an ACTH synthetic analogue in short- and long-term observation in order to find possible immunological markers of seizure pharmacoresistance, of good response to ACTH treatment and of the ACTH antiseizure mechanism.

Material and methods

Participants

The inclusion criteria for the study group were: 1) the diagnosis of pharmacoresistant epilepsy according to the 2010 definition by the International League Against Epilepsy (ILAE) [12]; 2) clinician's decision to introduce treatment with the $ACTH_{1-24}$ analogue; 3) informed consent of the patient's caregivers.

The exclusion criteria were: an acute infection, based on both clinical symptoms and elevated infection markers such as high C-reactive protein (CRP), and steroid treatment in the last six months.

The inclusion criteria for the age-matched control groups were: 1) diagnosis of epilepsy with full seizure control on the first antiepileptic drug monotherapy or history of seizure without the need of treatment (control group 1); 2) no history of any kind of seizure episodes (control group 2).

The exclusion criteria were the same as for the study group: an acute infection, based on both clinical symptoms and elevated infection markers such as high CRP, and steroid treatment in the last six months.

ACTH treatment management

The general ACTH management schedule used for the treatment of pharmacoresistant seizures in our hospital is as follows:

– first week is an inpatient treatment: intramuscular injections, in individuals weighing > 10 kg the dose is 0.5-1 mg/day, in individuals weighing < 10 kg body mass the daily dose is 0.25-0.50 mg. The patients are observed during their stay on the ward, with a special

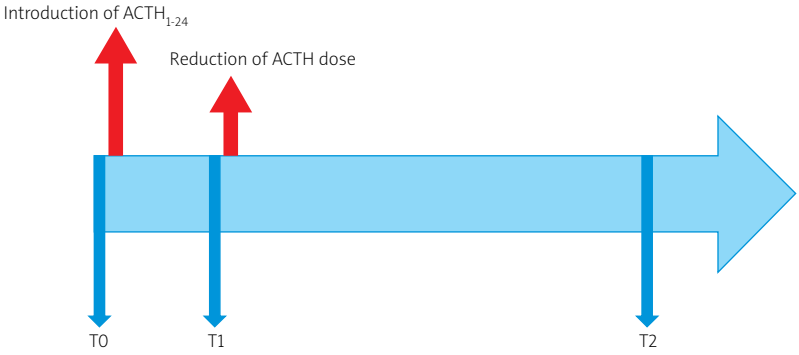

Fig. 1. Study design. Time T0 is the time of the basal cytokine concentration measurement, before the first dose of ACTH. Time T1 is the first cytokine level measurement on ACTH treatment. Time T2 is the second cytokine level measurement after ACTH initiation.

emphasis on seizure control and signs and symptoms of side effects;

– tapering (outpatients): usually lasts up to 3 months. It starts with the same doses as during the inpatient phase, but the time intervals between the doses are extended, meaning an injection every 2 days, then every 3 days, every 4 days, etc., down to one injection every 1-2 weeks.

Study design

The study started with blood sampling on the first day of ACTH treatment, before the first ACTH injection (T0). The second blood sampling (T1) took place at the end of the first week of ACTH treatment, before the tapering schedule. The third, last blood sampling was performed at the end of the tapering schedule. The study design is shown in Figure 1.

Data collected

The data collected during the study were: the age of the patient, sex, past medical history, duration of the epilepsy, etiology of epilepsy, seizure morphology and seizure number including the presence of infantile spasms, electroencephalographic (EEG) characteristics in all the three time points, pharmacological drug treatment except ACTH and concentrations of immunological markers at the three time points as mentioned earlier.

The following cytokines were analyzed: IL-1β, IL-1Ra (interleukin 1 receptor antagonist), IL-6, IL-8, IL-10, TNF-α, IFN-γ, MCP-1 (monocyte chemoattractant protein 1/CCL-2 – chemokine ligand 2) and MIP-1α (CCL3 – chemokine ligand 3).

In the control groups we collected demographic data and one sample of blood for cytokine analysis.

Additionally we collected information on the body mass, body mass index (BMI) at T0 and T2, CRP concentration and ACTH dose (daily and cumulative between T0 and T1 and between T0 and T2).

Cytokine concentration analysis

Immunological tests were performed at the Department of Biochemistry, Radioimmunology and Experimental Medicine, the Children's Memorial Health Institute, Warsaw, Poland. The plasma concentrations of IL-1β, IL-1Ra, IL-6, IL-8, IL-10, TNF-α, IFN-γ, MCP-1, MIP-1α were measured using commercially available, specific enzyme-linked immunosorbent assay (ELISA) kits (RayBiotech, Inc, Peachtree Corners, USA for MIP-1α, and BioVendor – Laboratorní medicína a.s., Brno, Czech Republic for the rest of parameters). The limit of detection (LoD) was 0.4 pg/ml, 30 pg/ml, 0.32 pg/ml, 0.5 pg/ml, 1.0 pg/ml, 2.3 pg/ml, 0.99 pg/ml, 2.3 pg/ml, and 6.0 pg/ml for IL-1β, IL-1Ra, IL-6, IL-8, IL-10, TNF-α, IFN-γ, MCP-1, MIP-1α, respectively. The overall intra-assay and inter-assay coefficient of variation (CV) was respectively: 4.2% and 6.7%, 7.2% and 7.4%, 5.1% and 5.0%, 5.2% and 8.2%, 3.2% and 5.6%, 6.0% and 7.4%, 4.5% and 5.7%, 4.7% and 8.7%, 10% and 12% for all of the above parameters. In the IL-1β and IL-8 assays the recombinant human IL-1β and IL-8 produced in *E. coli* were used as the standards (17.3 kDa protein consisting of 153 amino acid residues and 8.4 kDa protein consisting of 72 amino acid residues, respectively). Interleukin 6 standard used in the ELISA kit was calibrated against WHO 1st International Standard 89/548. The IL-10 and IFN-γ immunoassays were calibrated with highly purified recombinant human IL-10 and human IFN-γ, which have been evaluated against the international Reference Standard NIBSC 93/722 and 82/587, respectively (1 IU NIBSC 93/722 corresponding to 200 pg of human IL-10 and 1 IU NIBSC 82/587 corresponding to 50 pg of human IFN-γ).

Statistica for Windows software was used for statistical analysis. Basic data were analyzed with the descriptive statistics module and parameters such as mean, median, SD, and quartile range were calculated.

The Mann-Whitney *U* test (nonparametric alternative to the *t*-test for independent samples) was used to compare two different groups or subgroups at the same time point.

The Wilcoxon matched pairs test (nonparametric alternative to the *t*-test for dependent samples) was used to compare data in the analyzed group or subgroup at different time points.

The study has been approved by the local Ethics Committee (decision number 36/KBE/2012).

Results

From November 2014 to October 2018 we prospectively recruited for the study group 53 children, who presented at our department and who fulfilled the inclusion criteria. The most important demographic data of the study group and both control groups are presented in Table I. There were no statistically significant differences regarding the age at blood sampling between the two control groups or between the control groups and the study group.

Characteristics of epilepsy etiology in the study group are presented in Table II.

Seizure morphology in the study group with particular emphasis on infantile spasms is presented in Table III.

The majority (86%) of patients in the study group presented with West syndrome (infantile epileptic spasms syndrome – IESS), with the minimum of 2 major criteria of the syndrome [35]. The participants were on 1-5 antiepileptic medications at the time of the beginning of the study. Most of them (> 80%) were on 2-3 drugs. Valproic acid (VPA), vigabatrin (VGB) and levetiracetam (LEV) were the most commonly used in our study group.

The results of cytokine analyses in all the groups are shown in Tables IV-VII.

The only statistically significant difference in the cytokine concentration between the two control groups was with MCP-1, with higher levels in control group 2 – the one with no history of seizures. It is worth noting that cytokine levels in both the control groups were sometimes highly variable, with standard deviations close to or even higher than the mean and the median values, just to mention IL-1 and IL-6 as examples.

Comparing the cytokine levels at baseline (T0) in the study group to the control groups we found a strong statistically significant difference between the concentrations of MIP-1α (see Table V). A less strong statistically significant difference was found in concentrations of IL-1 and IL-8 between the two groups. Those differences could be a marker of seizure pharmacoresistance.

Table I. Demographic data of the study group and control groups

Parameter	Study group	Control groups (groups 1 and 2)	Control group 1	Control group 2
Number of individuals	53	32	11	21
Sex (F/M)	26/27	9/23	2/9	7/14
Age at T0 in months (mean/median)	3-80* (28.5/18)	1-65* (27/23)	10-65** (28/19)	1-65** (26/27)
Duration of epilepsy at T0 in months (mean/median)	0-74 (18.6/9.5)	–	–	–

**p = 0.67, **p = 0.87*

Table II. Characteristics of epilepsy etiology in the study group

Etiology of epilepsy	*n* (%)*
Genetic	19 (36)
Perinatal insult (prematurity, hypoxic-ischemic encephalopathy)	17 (32)
Structural changes in the MRI image	14 (26)
Postinflammatory	3 (5.6)
Other known	1 (1.9)
Unknown	23 (43)

**There were patients with more than one possible cause of epilepsy (e.g. genetic, such as tuberous sclerosis, and MRI changes)*

Table III. Seizure characteristics in the study group

Seizure morphology	Patient number	*N* = 52
Infantile spasms, *n* = 37 (71%)	Infantile spasms only	10
	Infantile spasms "plus"*	27
Other than infantile spasms, *n* = 15 (29%)	Focal motor only	6
	Generalized only	4
	Generalized and focal	5

**Patients with infantile spasms and other types of seizures*

Table IV. Cytokine characteristics in the control groups

Cytokine (pg/ml)	Control group 1			Control group 2			*p*-value
	Mean	Median	SD	Mean	Median	SD	
IL-1	2.17	0.86	4.05	1.18	0.78	1.25	NS
IL-6	17.68	1.79	43.6	11.58	4.54	20.55	NS
IL-8	2.63	2.46	0.75	2.71	2.14	1.19	NS
IL-10	5.52	3.22	7.94	3.77	3.10	2.7	NS
IL-1Ra	372.5	269.5	250.02	366.61	320.3	221.67	NS
TNF-α	2.81	2.68	0.48	2.63	2.51	0.50	NS
IFN-γ	9.37	1.32	15.44	7.29	2.09	8.74	NS
MCP-1	296.84	271.62	89.22	408.45	404.78	125.85	< 0.01
MIP-1α	6.90	6.82	0.41	7.11	6.92	0.77	NS

Table V. Cytokine characteristics at baseline (T0)

Cytokine (pg/ml)	Study group at T0			Control group 1 and 2			*p*-value
	Mean	Median	SD	Mean	Median	SD	
IL-1	2.81	0.75	11.49	1.52	0.79	2.55	< 0.01
IL-6	7.08	2.94	12.54	13.67	2.73	29.90	NS
IL-8	5.31	3.75	5.61	2.68	2.19	1.05	0.05
IL-10	9.28	3.41	26.13	4.37	3.16	5.07	NS
IL-1Ra	359.41	279.63	390.65	368.65	319.51	227.76	NS
TNF-α	2.77	2.42	1.14	2.69	2.54	0.49	NS
IFN-γ	13.29	2.66	20.53	8.00	1.74	11.27	NS
MCP-1	400.46	342.94	231.70	370.08	345.64	125.25	NS
MIP-1α	19.65	10.50	32.52	7.04	6.90	0.67	< 0.001

Table VI. Cytokine characteristics in the study group at T0 and T1

Cytokine (pg/ml)	Study group at T0			Study group at T1			*p*-value
	Mean	Median	SD	Mean	Median	SD	
IL-1	2.81	0.75	11.49	3.53	0.76	16.14	NS
IL-6	7.08	2.94	12.54	3.01	1.28	3.91	< 0.001
IL-8	5.31	3.75	5.61	4.79	3.83	2.86	NS
IL-10	9.28	3.41	26.13	9.65	2.92	26.37	NS
IL-1Ra	359.41	279.63	390.65	359.7	313.59	738.40	NS
TNF-α	2.77	2.42	1.14	2.56	2.41	0.58	NS
IFN-γ	13.29	2.66	20.53	8.13	1.77	12.52	< 0.05
MCP-1	400.46	342.94	231.70	204.24	154.55	124.70	< 0.001
MIP-1α	19.65	10.50	32.52	19.33	10.80	27.92	NS

Interestingly, the MCP-1 level in the study group at baseline (T0) was closer to the levels in = control group 2 than to the levels in control group 1 (Tables IV and V).

Comparing the cytokine levels in the study group at T0 and T1, we found a strong statistically significant difference between the concentrations of IL-6, IFN-γ and MCP-1 (Table VI). The concentration of IL-6 in the study group at T1 was significantly lower as compared to the level at T0, and was even much lower when compared to the IL-6 levels in both the control groups. The concentration of IFN-γ in the study group at T1 was also significantly lower as compared to the level at T0, and reached a similar level as IFN-γ in both the control groups. This is an effect of the anti-inflammatory ACTH mechanism. The MCP-1 change in concentration from T0 to T1 made it similar to the difference between the

Table VII. Cytokine characteristics in the study group at T0 and T2

Cytokine (pg/ml)	Study group at T0			Study group at T2			*p*-value
	Mean	Median	SD	Mean	Median	SD	
IL-1	2.81	0.75	11.49	2.077	0.64	4.68	NS
IL-6	7.08	2.94	12.54	7.01	2.62	11.02	NS
IL-8	5.31	3.75	5.61	4.58	4.34	2.60	NS
IL-10	9.28	3.41	26.13	7.55	3.38	12.65	NS
IL-1Ra	359.41	279.63	390.65	342.37	245.26	459.59	NS
TNF-α	2.77	2.42	1.14	2.57	2.32	0.64	NS
IFN-γ	13.29	2.66	20.53	14.75	3.19	23.13	NS
MCP-1	400.46	342.94	231.70	381.19	367.83	196.47	NS
MIP-1α	19.65	10.50	32.52	29.08	11.61	30.03	NS

MCP-1 concentration in control groups 2 and 1. That could be the probable mechanism of the ACTH effect.

No statistically significant differences were found between cytokine concentrations at T0 and T2 in the study group (see Table VII). The IL-6 concentrations had returned to the baseline T0, meaning the anti-inflammatory ACTH effect is not significant in this aspect when the drug is withdrawn. The MCP-1 concentrations similarly to IL-6 also increased from T1 to T2, reaching values close to those at T0.

In the next step we analyzed the ACTH responsiveness.

The majority of patients responded to ACTH treatment. We considered a "responder" to be an individual who showed more than a 50% reduction of daily seizure number at T1 or at T2 as compared to at T0. A diagram presenting the course of our observations is presented in Figure 2. There were 4 children who turned from a non-responder at T1 to a responder at T2.

We analyzed the responders and non-responders to ACTH at T1 in all the available variables. We found no statistically significant difference in any variable between the groups. There was no statistically significant difference between the responders and non-responders at T1 in the use of standard antiseizure medications or the presence of hypsarrhythmia at T0. The most interesting quantitative data are shown in Table VIII.

We looked at possible immunological markers of response at T1 in the study group. The results are shown in Tables IX-X.

There was no difference between responder and non-responder groups in the level of any cytokine, either in T0 or in T1 samples (Table IX).

We found statistically significant differences in MCP-1 concentrations in both responder and non-responder groups between T0 and T1. Additionally, there was a significant change of IL-6 in the responder group and of IFN in the non-responder group. All the markers decreased from T0 to T1.

We performed the same statistical analysis for the observation at time point T2. As for the T1 analysis there was no statistically significant difference between the responders and non-responders at T2 in the use of standard antiseizure medications or the presence of hypsarrhythmia at T0.

We found a statistically significant difference between groups for the ACTH cumulative dose at T2 (Table XI). In the responder group this dose was signifi-

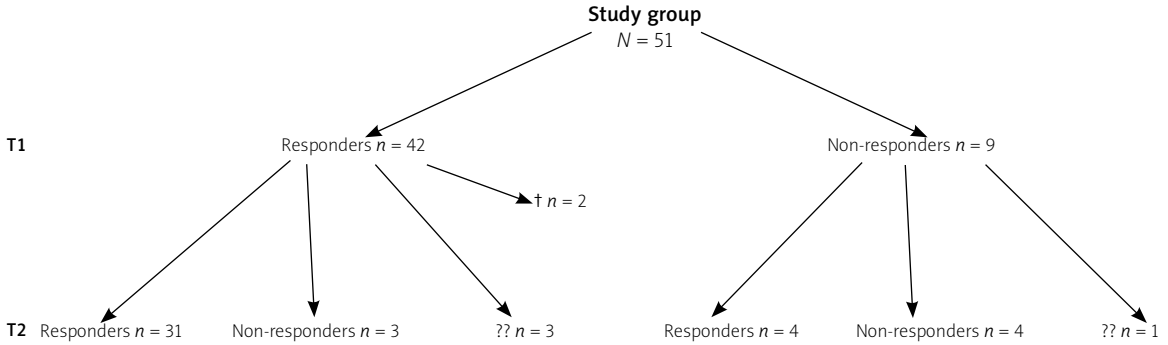

Fig. 2. Responders and non-responders number and distribution at T1 and T2.

Table VIII. Characteristics of responders and non-responders at T1

Parameter	Study group responders at T1			Study group non-responders at T1			*p*-value
	Mean	Median	SD	Mean	Median	SD	
Age at T0 in months	31.7	27.0	25.4	17.0	13.0	12.2	NS
Duration of disease*	20.6	11.5	21.8	9.6	7.0	9.7	NS
Body weight (kg) at T0	11.5	10.7	3.9	10.4	10.7	3.2	NS
BMI's percentile at T0	28.0	25.0	21.9	45.5	25.0	37.9	NS
ACTH dose‡	0.38	0.32	0.18	0.44	0.22	0.48	NS

**In months, ‡ACTH cumulative dose from T0 to T1 in mg/kg*

Table IX. Cytokine characteristics in responders and non-responders at T1

Cytokine (pg/ml)	Study group responders at T1			Study group non-responders at T1			*p*-value
	Mean	Median	SD	Mean	Median	SD	
IL-1 at T0	3.32	0.76	12.92	0.69	0.66	0.09	NS
IL-1 at T1	4.04	0.75	17.59	0.82	0.77	0.15	NS
IL-6 at T0	7.86	3.47	13.89	3.40	2.34	3.10	NS
IL-6 at T1	3.16	1.32	4.18	2.25	1.24	2.06	NS
IL-8 at T0	4.60	3.51	3.49	4.90	4.09	2.61	NS
IL-8 at T1	5.16	4.25	2.99	2.91	2.81	0.48	NS
IL-10 at T0	10.86	3.30	29.29	3.16	3.52	1.37	NS
IL-10 at T1	11.04	2.93	28.68	2.53	2.48	1.15	NS
IL-1Ra at T0	376.91	282.26	430.88	289.68	261.82	187.01	NS
IL-1Ra at T1	498.89	342.43	798.73	258.35	253.05	196.69	NS
TNF-α at T0	2.75	2.42	0.92	3.00	2.42	2.00	NS
TNF-α at T1	2.61	2.42	0.62	2.30	2.32	0.17	NS
IFN-γ at T0	12.06	2.51	20.68	16.75	3.94	19.62	NS
IFN-γ at T1	7.46	1.67	11.70	11.60	3.62	16.79	NS
MCP-1 at T0	407.27	329.45	257.32	371.22	360.16	88.45	NS
MCP-1 at T1	207.48	156.73	133.34	188.03	174.80	71.66	NS
MIP-1α at T0	20.70	10.90	34.77	17.38	7.90	25.53	NS
MIP-1α at T1	18.61	10.83	26.52	23.03	9.37	36.51	NS

cantly lower. We consider that this higher dose in the non-responder group is a marker of resistance to treatment – in this group the doses of ACTH were maintained higher or even increased during treatment and the number of injections was higher in order to reach seizure control. However, this higher cumulative dose did not result in weight gain in the non-responder group.

The results of the cytokine concentration analysis are summarized in Table XII.

We found no statistically significant differences between the responder and non-responder groups at T2 except for IL-8 at T2. In the non-responder group the concentrations of IL-8 at T2 were significantly lower than in the responder group. What is worth noting is that IL-8 concentration at T2 in the non-responder group had values close to the control group (Tables V and XII).

It was interesting to note that the concentration of IFN-γ before ACTH treatment (T0) seemed lower in the non-responder group at T2 than in the responder group. That would be a marker of ACTH resistance to treatment before the treatment. This difference was far from statistical significance (Table XII).

We also looked at a possible relationship between the most commonly used antiepileptic drugs (AED) in our study group and the cytokine concentrations. The medications considered were: VGB, VPA and LEV.

The statistically significant results are shown in Table XIII.

The concentrations of IL-10 and IFN-γ were significantly lower in the group receiving LEV. The concentration of TNF-α was higher in the group receiving VPA and the concentration of MIP-1α was lower in the group receiving VPA.

Table X. Cytokine change from T0 to T1 in the responders and non-responders at T1

	Cytokine (pg/ml)	Concentration at T0			Concentration at T1			*p*-value
		Mean	Median	SD	Mean	Median	SD	
Responders at T1	IL-1	3.32	0.76	12.92	4.04	0.75	17.59	NS
	IL-6	7.86	3.47	13.89	3.16	1.32	4.18	< 0.01
	IL-8	4.60	3.51	3.49	5.16	4.25	2.99	NS
	IL-10	10.86	3.30	29.29	11.04	2.93	28.68	NS
	IL-1Ra	376.91	282.26	430.88	498.89	342.43	798.73	NS
	TNF-α	2.75	2.42	0.92	2.61	2.42	0.62	NS
	IFN-γ	12.06	2.51	20.68	7.46	1.67	11.70	NS
	MCP-1	407.27	329.45	257.32	207.48	156.73	133.34	< 0.001
	MIP-1α	20.70	10.90	34.77	18.61	10.83	26.52	NS
Non-responders at T1	IL-1	0.69	0.66	0.09	0.82	0.77	0.15	NS
	IL-6	3.40	2.34	3.10	2.25	1.24	2.06	NS
	IL-8	4.90	4.09	2.61	2.91	2.81	0.48	NS
	IL-10	3.16	3.52	1.37	2.53	2.48	1.15	NS
	IL-1Ra	289.68	261.82	187.01	258.35	253.05	196.69	NS
	TNF-α	3.00	2.42	2.00	2.30	2.32	0.17	NS
	IFN-γ	16.75	3.94	19.62	11.60	3.62	16.79	0.01
	MCP-1	371.22	360.16	88.45	188.03	174.80	71.66	0.01
	MIP-1α	17.38	7.90	25.53	23.03	9.37	36.51	NS

Table XI. Characteristics of responders and non-responders at T2

Parameter	Study group responders at T2			Study group non-responders at T2			*p*-value
	Mean	Median	SD	Mean	Median	SD	
Age at T0 (months)	25.31	16.00	21.92	34.28	28.00	27.31	NS
Duration of disease (months)	13.71	5.00	16.06	26.28	20.00	27.03	NS
Body weight (kg) at T0	10.90	10.16	3.89	11.87	10.90	3.58	NS
BMI's percentile at T0	32.17	25.00	23.37	31.85	10.00	35.48	NS
BMI's percentile at T2	57.38	50.00	28.68	11.60	10.00	8.08	0.001
ACTH dose‡	0.39	0.32	0.24	0.44	0.31	0.31	NS
ACTH dose§	1.09	1.04	0.46	1.93	1.47	1.01	0.05

‡ACTH cumulative dose from T0 to T1 in mg/kg, §ACTH cumulative dose from T0 to T2 in mg/kg

Some side effects of ACTH treatment have been observed in our study group. The most common were upper and lower respiratory tract infections (in 15/53 patients), sometimes resulting in a change in the ACTH administration regime. Another frequently reported complication was a negative change in mood/behavior (12/53 patients). This change was reported as high irritability and sleep disturbances. In contrast, in 6/53 patients the change of behavior was described by caregivers as positive. For them it meant improvement in social responsiveness and mood stabilization. Less common treatment complications were elevated blood pressure (5/53), hyperglycemia (3/53) and in one case adrenal insufficiency secondary to ACTH.

Discussion

The aim of our study was to find possible immunological markers of seizure pharmacoresistance, possible markers of good response to ACTH synthetic analogue treatment and potential immunological pathways of the ACTH antiseizure effect.

We found statistically significantly higher concentrations of IL-1, IL-8 and MIP-1α at baseline (T0) in the study group compared to the control groups. Few

Table XII. Cytokine characteristics in the responders and non-responders at T2

Cytokine (pg/ml)	Study group responders at T2			Study group non-responders at T2			*p*-value
	Mean	Median	SD	Mean	Median	SD	
IL-1 at T0	3.63	0.76	13.99	0.68	0.61	0.11	NS
IL-1 at T2	2.27	0.65	4.97	0.62	0.62	0.02	NS
IL-6 at T0	7.61	3.01	14.82	3.51	2.34	3.55	NS
IL-6 at T2	7.79	3.10	11.55	1.27	1.15	0.63	NS
IL-8 at T0	4.83	3.75	3.56	4.12	2.96	3.01	NS
IL-8 at T2	4.93	4.70	2.60	2.11	2.08	0.19	0.05
IL-10 at T0	11.35	3.20	31.59	3.54	3.52	2.05	NS
IL-10 at T2	7.95	3.65	13.40	4.62	2.14	4.60	NS
IL-1Ra at T0	313.95	261.82	415.47	338.91	270.63	157.61	NS
IL-1Ra at T2	348.83	244.13	490.09	294.99	251.40	94.39	NS
TNF-α at T0	2.71	2.39	1.17	2.43	2.30	0.43	NS
TNF-α at T2	2.59	2.33	0.67	2.47	2.28	0.43	NS
IFN-γ at T0	12.22	2.57	20.06	5.40	1.97	6.33	NS (0.81)
IFN-γ at T2	14.80	3.64	23.76	14.33	2.36	21.91	NS
MCP-1 at T0	424.36	325.21	270.61	346.96	360.16	117.36	NS
MCP-1 at T2	389.5	368.47	205.80	319.84	257.79	109.89	NS
MIP-1α at T0	19.35	10.93	31.60	15.61	7.90	18.58	NS
MIP-1α at T2	28.52	11.64	29.86	33.14	11.42	37.87	NS

Table XIII. Cytokine characteristics and antiepileptic medication use

Cytokine (pg/ml)	Mean	Median	SD	Mean	Median	SD	*p*-value
	Study group LEV+			Study group LEV–			
IL-10 at T0	4.35	2.47	7.44	13.60	4.09	36.18	0.05
IFN-γ at T0	5.21	1.22	7.35	15.02	3.80	22.28	< 0.05
	Study group VPA+			Study group VPA–			
TNF-α at T0	2.94	2.51	1.40	2.33	2.27	0.29	< 0.05
MIP-1α at T0	13.18	9.38	13.75	27.43	11.31	43.12	< 0.05

LEV – levetiracetam, VPA – valproic acid. LEV+ means cytokine concentration in patients receiving levetiracetam, LEV– means cytokine concentration in patients not receiving levetiracetam, VPA+ means cytokine concentration in patients receiving valproic acid, VPA– means cytokine concentration in patients not receiving valproic acid

papers are available on immunological markers during ACTH treatment in epilepsy. In a study from a Turkish group 20 children with infantile spasms were observed during ACTH synthetic analogue treatment together with 20 healthy controls [27]. The concentrations of IL-1β, IL-2, IL-6, IL-17, IL-23 and TNF-α were measured at the beginning of ACTH treatment and a month after completion of 11 injections of the drug. They found a significant difference in IL-6 level between the pre-treatment study group and the control group, with much lower levels in the control group. This observation is contradictory to our results, as we observed a lower mean level of IL-6 in our pretreatment study group compared to controls; however, due to the great variation in concentration of this cytokine (high SD values), this difference was not statistically significant. In contrast, in the pre- and post-treatment analysis of our work we observed a statistically significant reduction of IL-6 (T0-T1 interval), as in the Turkish group; however, this time their research did not show statistical significance. We postulate that high concentrations of IL-1, IL-8 and MIP-1α could mark difficult to treat epilepsy in children.

Our research did not let us identify any reliable immunological marker of response to ACTH treatment. Lower IFN-γ levels at baseline (T0) in the non-responders at T2 did not reach statistical significance.

We did not observe a positive effect of higher ACTH doses on the response rate in our patients. This is further proof that higher doses of ACTH do not mean a better treatment result.

Adrenocorticotropic hormone significantly reduced levels of IL-6, IFN-γ and MIP-1α in the intensive treatment period (T1), but in all three markers this effect

was short lasting and no significant changes in cytokine levels were found between T2 and T0. Steinborn *et al*. looked at changes in levels of certain cytokines (IL-1β, IL-2, IL-6, TNF-α) in a group of patients with newly diagnosed generalized epilepsy treated with VPA [25]. They found significant reduction of IL-6 after 4-6 months of VPA supply. Lowering IL-6 might be a common finding in the VPA and ACTH antiseizure mechanism of action.

Guenther *et al*. in their study looked at the blood concentrations of IL-1β, IL-6, TNF-α and MCP-1 in patients above 15 years of age and with newly diagnosed epilepsy treated with VPA or LEV [8]. They did not find any significant change in the analyzed cytokines after introducing those medications. Labh *et al*. analyzed blood MCP-1 (CCL2) and IL-1β concentrations in children with newly diagnosed epilepsy and VPA only treatment (group 1) and with epilepsy treated with both VPA and LEV as add on due to uncontrolled seizures on VPA alone (group 2) [13]. After treatment modification in both groups they observed a good response (> 50% reduction of seizures) in more than 90% of patients in both groups. The change in IL-1β was not statistically significant. A statistically significant decrease in MCP-1 in both the valproate only group and the valproate and levetiracetam group was observed after 16 weeks. The authors noted that the baseline MCP-1 in group 2 was significantly higher than the MCP-1 measured after 16 weeks in group 1 and concluded that a high MCP-1 concentration is related to bad seizure control. In our research we also observed significant lowering of MCP-1 after the treatment modification (from T0 to T1). This MCP-1 change however was not durable. The MCP-1 concentration at T0 also did not significantly differ to MCP-1 in the control groups. In addition, the MCP-1 level at T1 was not significantly different in the responder and non-responder groups. We concluded that the reduction of MCP-1 is attributable to the ACTH anti-inflammatory mechanism of action and not to the seizure load.

Our study showed significantly lower concentrations of IL-10 and IFN-γ in the group of patients receiving levetiracetam. The concentration of TNF-α was higher and the concentration of MIP-1α was lower in the group receiving VPA. Our results together with the already mentioned published papers indicate that classical antiseizure medications interact with the immunological system. Whether this could be an additional mechanism of AED antiseizure effect needs more exploration.

Conclusions

Our study found three possible immunological markers of pharmacoresistance in epilepsy: IL-1, IL-8 and MIP-1α. Moreover, our results suggest that IL-6, IFN-γ and MCP-1 may play a role in the immunological effect of ACTH treatment. Our findings indicate that classical antiseizure drugs might also partially act through immunological mechanisms.

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Disclosure

The authors report no conflict of interest.

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