

## research article

# Dynamic susceptibility contrast enhanced (DSC) MRI perfusion and plasma cytokine levels in patients after tonic-clonic seizures

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**Background.** Inflammatory events in brain parenchyma and glial tissue are involved in epileptogenesis. Blood concentration of cytokines is shown to be elevated after tonic-clonic seizures. As a result of inflammation, blood-brain barrier leakage occurs. This can be documented by imaging techniques, such is dynamic susceptibility contrast enhanced (DSC) MRI perfusion. Our aim was to check for postictal brain inflammation by studying DSC MRI perfusion and plasma level of cytokines. We looked for correlations between number and type of introducing seizures, postictal plasma level of cytokines and parameters of DSC MRI perfusion. Furthermore, we looked for correlation of those parameters and course of the disease over one year follow up.

**Patients and methods.** We prospectively enrolled 30 patients, 8–24 hours after single or repeated tonic-clonic seizures.

**Results.** 25 of them had normal perfusion parameters, while 5 had hyperperfusion. Patients with hyperperfusion were tested again, 3 months later. Two of 5 had hyperperfusion also on control measurements. Number of index seizures negatively correlated with concentration of proinflammatory cytokines IL-10, IFN- $\gamma$  and TNF- $\alpha$  in a whole cohort. In patients with hyperperfusion, there were significantly lower concentrations of antiinflammatory cytokine IL-4 and higher concentrations of proinflammatory TNF- $\alpha$ .

**Conclusions.** Long lasting blood- brain barrier disruption may be crucial for epileptogenesis in selected patients.

Key words: seizure; blood-brain barrier; cytokines; DSC MRI

## Introduction

Epilepsy is a chronic disorder characterized by the recurrence of unprovoked seizures. The consequences of epilepsy are not only neurobiological but also cognitive, physiological and social.<sup>1</sup> Epilepsy affects roughly 50 million people worldwide.<sup>2</sup> One third of all affected persons suffer from intractable seizures. With the exception of surgical resection, in most instances treatment is only symptomatic.<sup>3</sup>

A single seizure is not yet epilepsy; it may be a symptom of infection, trauma, stroke, metabolic derangements etc., and may never repeat.<sup>4</sup>

The crucial process leading to repeated seizures is epileptogenesis; a process from initial brain damage to the first seizure and beyond, consisting of a sequence of chemical and structural alterations resulting in transformation of normal to epileptogenic brain tissue. Epileptogenesis may last years.<sup>5</sup> The initial event can be a seizure per se.<sup>6,7</sup>

Indices of inflammation in epilepsy have been repeatedly demonstrated but there is no clear answer as to whether inflammation is the cause or a consequence of the disease.<sup>8,9</sup> There is evidence that a single seizure promotes a surge of cytokine production in brain tissue.<sup>10,11</sup> Repeated seizures

tend to be accompanied by a higher level of brain cytokine production.<sup>12</sup> Elevated peripheral blood levels of cytokines, mostly interleukin-6 (IL-6) and antagonist of IL-1 Receptor (IL-1Ra) have been reported after secondarily generalized<sup>13</sup> and focal epileptic seizures.<sup>14,15</sup> Blood perfusion changes have so far been extensively studied in patients with acute ischemic stroke<sup>16,17</sup>, and there have also been studies in patients with epilepsy.<sup>18,19</sup> In animal studies, early brain perfusion changes after status epilepticus have been suggested to be of prognostic value in relation to future hippocampal shrinkage and degeneration.<sup>20,21</sup> Vascular perfusion changes were not localized but spread throughout wide brain areas; in a model of prolonged focal seizures, the effect was explained by diffuse changes in neurovascular coupling.<sup>22,23</sup> Changes in cerebral blood perfusion related to seizures have also been attributed to higher metabolic demand<sup>24</sup> and leucocyte-endothelial interaction.<sup>25</sup> In humans, assessment of perfusion changes after a single seizure<sup>26</sup> or status epilepticus<sup>27</sup> has been reported to be helpful in localizing the epileptic focus.

It was our aim to check for postictal brain inflammation indices by studying MR perfusion and blood cytokines, and to determine whether the type and number of index seizures, postictal blood concentrations of cytokines and parameters of MR perfusion are predictive for the seizure burden over a one year follow-up.

## Patients and methods

### Patients

We *prospectively* enrolled 30 patients presenting in the Emergency Service of the Division of Neurology, University Medical Centre Ljubljana, Slovenia after one or multiple seizures, from 1. 6. 2011 to 31. 12. 2014.

*Inclusion criteria were:* one or more witnessed seizures in the last 24 hours, age 18–65 years, informed written consent for study enrolment and informed written consent for the contrast MRI examination. *Exclusion criteria were:* brain injury, haemorrhage or stroke in the last 6 months, primary or secondary brain tumour any time in the past, known autoimmune disease or any immune modifying therapy in the last 6 months, pregnancy or lactation, alcohol or drug abuse, liver or kidney failure, clinical signs of infection or CRP > 20 (mg/l) and inability to obtain informed written consent (dementia, mental retardation, altered consciousness, psychosis).

The study was approved by the National Medical Ethics Committee.

### Study protocol

Written consent was signed; a full history was recorded, including smoking status. Patients were given an Epilepsy Diary with instructions for its use. A minimum 8 and maximum 24 hours after the last seizure, 2 ml of venous blood in EDTA buffer were taken and transported for immediate processing. C-reactive protein (CRP) was checked on a Point of Care device. Brain MRI with contrast was performed. Routine 32-channel EEG with scalp electrodes was recorded. Patients with reported »hyperperfusion« after visual MRI analysis were invited for a control blood and MRI examination after three months. One year after the index event, patients were contacted by phone and relevant information about disease activity was collected.

Individual researchers (clinicians, immunologist and radiologist) were unaware of the results of investigations performed by other researchers in the course of the study.

### Assessment of clinical and EEG data

The severity of the disease in the year prior to the study was assessed by the National Hospital Seizure Severity (NHS) 3 score<sup>28</sup>; if two types of seizures were reported, the »lesser« one (including aura) being chosen first. The frequency of seizures in the year prior to the study was defined as: 1. Rare (R) < 1 seizure per year, 2. Often (O) > 1 seizure per year and 3. Very often (V) > 1 seizure per month.

EEG findings were categorized as: 1. Normal, 2. Focal changes (location included), 3. Generalized changes and 4. Nonspecific abnormalities. Syndromological classification was performed based on International League Against Epilepsy (ILAE) criteria.<sup>1,29</sup> The category »probable focal symptomatic« was used if the semiology of the seizures strongly suggested focal onset (*e.g.*, »déjà vu«, head turn) in the presence of generalized or unspecific EEG changes. Generalized changes were categorized either as IGE (*e.g.* multiple spikes/waves) or nonspecific generalized (*e.g.* short generalized delta/theta rhythm).

### Immunological methods

The concentrations of interleukins: 4 (IL-4), 6 (IL-6), 10 (IL-10) and 12 (IL-12), antagonist of IL-1 receptor (IL-1Ra), tumour necrosis factor- $\alpha$  (TNF- $\alpha$ ) and

interferon  $\gamma$  (IFN- $\gamma$ ) were measured. The concentration of high sensitivity CRP was used additionally to rule out acute infections and autoimmune disorders.

Two ml of blood were taken in a test tube recoated with ethylenediaminetetraacetic acid (EDTA), immediately centrifuged and stored until processing at  $-20^{\circ}\text{C}$ . A chemoluminescent immunometry assay based on tagged monoclonal antibodies was performed. In brief, the system adds serum and reagent (cytokine specific antibody conjugated with alkaline phosphatase) into a test tube containing the carrier covered with cytokine specific antibody. The whole sample is then incubated until complex *cytokine specific antibody-cytokine-alkaline phosphatase conjugated cytokine specific antibody* is formed. The sample is then rinsed (to remove the non-bound reagent) and the luminogenous substrate is added. Alkaline phosphatase bound via cytokine to a carrier splits the substrate into an unstable luminescent intermediary. The level of luminescence corresponds to the serum level of cytokine. The test was performed using the commercial Immulite 1000 and Immulite 2000 XPI systems (Siemens, Germany; detailed description available in: N CEL 19 and N CEL-18 Siemens Manual). Controls were performed with the commercial IMMULITE Cytokine Control Module (Siemens). The concentration of cytokine is calculated by a calibration curve generated by the manufacturer for each lot of reagents and saved at the bar code of the test kit. Reference values are given by the manufacturer for each cytokine and soluble receptor.

## MRI Methods

All MRI images were obtained on a 1.5T MRI scanner (Philips Achieva Nova, Netherlands). Morphological brain studies were obtained using the following sequence protocol: T1 weighted spin echo (SE), T2 weighted fast field echo (FFE) and diffusion weighted imaging (DWI) in transversal plane; fluid attenuated inversion recovery (FLAIR), T1 weighted inversion recovery (IR) echoplanar imaging (EPI) and T2 weighted fast spin echo (FSE) in coronal and transversal planes; T1 weighted three dimensional (3D) in sagittal plane. MR perfusion was performed by fast field echo (FFE) echo planar imaging (EPI) sequence.

Each subject obtained a 16 G cubital *i.v.* line. Gadolinium-based contrast agent (Gadovist; Bayer HealthCare Pharmaceuticals, Germany) was used in the standard dose (0.1 mmol/kg), with flow ve-

locity 4 ml/s. At the end of the contrast bolus, 20 ml of normal saline with the same flow was given. Contrast agent and normal saline were given by automatic injector. First-pass dynamic susceptibility-weighted contrast-enhanced perfusion-weighted imaging (DSC-MRI) was then performed. DSC-MRI was done using the planar echo 3D method: the signal from the whole k-plane was acquired after a single  $90^{\circ}$  radiofrequency pulse in order to reach maximum study speed. Data acquisition was done before, during and after contrast bolus (20 slices with 5 mm thickness, matrix size  $128 \times 128$  and size of examined field 224 mm). The repetition time (TR) was 1501 ms, the acquisition time for each dynamic volume was 1.6 s. The echo time (TE) was 40 ms, flip angle  $75^{\circ}$ . Each perfusion sequence consisted of 40 dynamic images, lasting 66 s for each first-pass.

First, two experienced neuroradiologists independently visually interpreted the morphological MRI sequences and then the functional colour maps of perfusion parameters. An automatic deconvolution program (Neuro T2\* perfusion package, Philips Achieva Nova, Netherlands), which is a part of the MRI scanner software, was used. The program automatically calculated the perfusion parameters: negative integral, *i.e.*, blood volume (BV), mean transit time (MTT), time to peak (TTP) and an index representing blood flow (BF). The deconvolution method is based on a predetermined arterial input function (AIF). AIF was set on the medial cerebral artery (MCA) at the side of the brain with larger diameter and more prominent flat course. According to methods previously described<sup>30, 31</sup>, we manually chose and marked two regions of grey matter with either higher blood flow or higher blood volume, so called regions of interest (ROIs), and calculated the relative values of perfusion parameters.

## Statistical methods

The commercially available SPSS version 21.0 program was used. All values are given as absolute (N) and percentage (%). Mean values are expressed as median (Me) and/or mean (M) with standard deviation (SD). The normality of the data was tested by Kolmogorov-Smirnov and Shapiro-Wilk tests. Since most of the data was not normally distributed, nonparametric statistical methods were used. Correlation was tested with Pearson's correlation and Eta coefficient. For group comparison, the Wilcoxon, Kruskal-Wallis, Chi-square and Mann-Whitney U tests were used.

## Results

Demographical and clinical data are summarized in Table 1, EEG and syndrome data in Table 2.

In four patients (13.3%) the index seizure was their first seizure; patient 3 had only had one seizure in the past.

None of the 30 patients had hypoperfusion on MRI, 25 had normal MRI perfusion parameters (»normoperfusion« patients); five (17%) (Patients 2, 3, 7, 15 and 25) had hyperperfusion (»hyperperfusion« patients) (Figure 1). All »hyperperfusion« patients had normal structural MRI; 9 of the remaining 25 patients with normal perfusion parameters had structural abnormalities. Two had more diffuse structural abnormalities: patient 1 had leucopathy, patient 28 had cerebellar atrophy. Patient 5 had had resection of the right frontal lobe in childhood; patient 26 had an aneurysm of the left medial cerebral artery. We did not find associations between structural MRI abnormalities and

either postictal cytokine level or MRI perfusion parameters. Patients 7 and 15 also had hyperperfusion also on the control MRI examination after three months (»control hyperperfusion«). During this period, both patients were seizure free (*i.e.*, the date of the last seizure was the date of enrolling in the study).

MRI parameters after index seizure(s) are shown in Table 3.

Blood samples were drawn 8 hours after the last seizure in most patients, except in patient 1 (24 h), patient 9 (13 h), patient 14 (15 h) and patient 16 (20 h). The blood sample of patient 3 was accidentally lost.

The tested cytokines, including concentrations of IL-6, showed marked variability.

Cytokine levels after the index seizure(s) are summarized in Table 4. Median concentrations of cytokines in the three subgroups of patients (»normoperfusion«, »hyperperfusion«, and »control hyperperfusion«) are provided in Table 5.

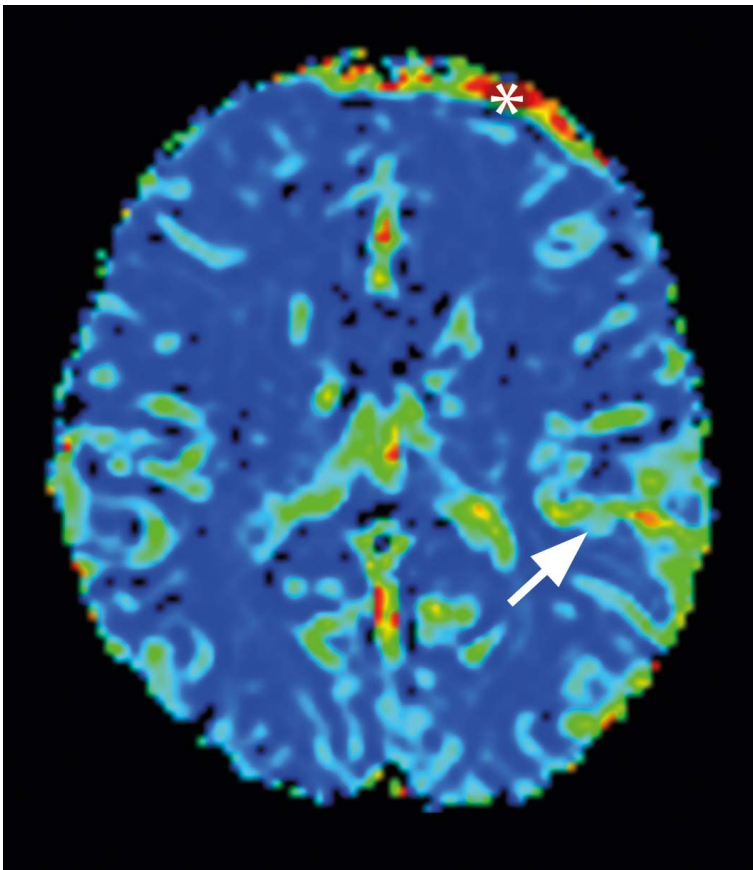
Postictal (1<sup>st</sup>) and control levels of cytokines in patients with hyperperfusion after the index seizure(s) are summarized in Table 6. The number of index seizures significantly negatively correlated with concentrations of IL-10, IFN- $\gamma$  and TNF- $\alpha$  (Table 7).

There was a significant correlation among concentrations of the following cytokines: IL-6 and IL-12 ( $\rho = 0.37$ ,  $p < 0.05$ ); TNF- $\alpha$  and hsCRP ( $\rho = 0.71$ ,  $p < 0.01$ ); TNF- $\alpha$  and IL-1Ra ( $\rho = 0.51$ ,  $p < 0.01$ ), and IL-1Ra and IFN- $\gamma$  ( $\rho = 0.39$ ,  $p < 0.05$ ).

Taking into account the whole cohort of patients, cytokine concentrations did not correlate with postictal MRI perfusion parameters. Postictal MRI perfusion parameters were furthermore not correlated with any other tested variable (age, gender, smoking status, number of index seizures, antiepileptic drug, epileptic syndrome, EEG, structural MRI or seizure frequency in the previous year).

Analysis was then performed for the two subgroups of patients - »normoperfusion« (25 patients), and »hyperperfusion« (5 patients). There were a slightly higher proportion of smokers in the »hyperperfusion« group: (60% *vs.* 40% in the »normoperfusion« group). The percentage of idiopathic generalized epilepsy was identical between the two groups (20%). Overall, 12 patients had focal EEG abnormalities but there was only one of these in the »hyperperfusion« group (left temporal changes).

In the »hyperperfusion« group, concentrations of most of the cytokines were lower but the difference reached significance only for IL-4 ( $Z = -1.98$ ;



**FIGURE 1:** increased blood flow (arrow) in the region of the left medial cerebral artery; signal changes adjacent to left frontal bone (asterisk) are the artefact (patient 15)

TABLE 1. Demographical and clinical data

	Sex age,	Smoking status	Illness Duration (yr)	syndrome	EEG	FREQUENCY	N-INDEX	AED	TYPE /NHS3	Structural MRI	Follow up
1	F 65	/	7	PFS	G	V	4	LAM, LEV	2(5/10)	Leucopathy	0
2	F 38	5	7	PFS	G	R	1	LEV*	2 (2/10)	N	0
3	M 26	/	3	/	N	R	1	LEV*	2(2/12)	N	0
4	F 65	/	/	/	G	1 <sup>ST</sup>	1	/	1(11)	N	0
5	F 23	/	15	FS	F (RF)	V	1	TPM, LAM, DPH	2 (2/12)	Resection RF	>100
6	M 35	/	20	FS	F (RF)	R	1	LAM	2(2/10)	N	0
7	M 42	/	1	FS	F(RT)	1 <sup>ST</sup>	1	LEV*	1(18)	N	5
8	F 20	10	7	IGE	G	O	1	VA	2(2/13)	N	0
9	F 24	/	1	/	N	2 <sup>nd</sup>	1	/	1(12)	N	0
10	M 21	20	6	PFS	N	O	1	LEV*	1(13)	N	0
11	M 29	/	12	IGE	G	O	1	VA	2(2/14)	N	0
12	M 46	/	/	FS	F(LT)	1 <sup>ST</sup>	1	LEV* PREG**	1(13)	Encephalomalacy LT	0
13	F 57	/	/	/	F(RF)	1 <sup>ST</sup>	1	/			
14	M 27	/	7	FS	F(LT)	R	1	LEV*	1(18)	N	6
15	F 54	/	8	PFS	N	O	1	LEV	1 (11)	N	0
16	M 44	10	5	FS	F(LF)	O	3	LAM	1(12)	N	0
17	F 45	/	20	PFS	N	R	1	LAM	1(15)	N	0
18	M 30	10	12	FS	F(RF)	O	1	LAM	1(18)	Gliosis RF	5
19	M 63	/	4	FS	F(RT)	O	5	LEV	2(2/18)	MTS R	12
20	M 40	/	20	FS	F(RT)	O	1	CBZ	2(2/15)	N	12
21	M 41	/	20	FS	F(LT)	V	5	CBZ,VA	1(10)	N	50
22	M 23	/	8	IGE	G	V	3	LAM	1(18)	N	47
23	F 29	/	11	FS	F(RT)	V	2	TPM,LAM	1(20)	Cortical. Dysplasia RT	5
24	M 27	/	12	IGE	G	O	7	VA*	1(9)	N	2
25	M 21	10	6	IGE	G	O	6	VA	2(2/8)	N	0
26	F 50	20	34	FS	F(LT)	R	1	/	1(8)	Aneurysm L MCA	0
27	F 43	/	25	PFS	N	V	3	LAM	1(11)	Gliosis RF	0
28	M 50	/	40	IGE	G	V	1	LAM,LEV	1(19)	Cerebellar atrophy	18
29	M 39	/	32	/	G	R	1	/	1(8)	N	0
30	M 19	10	5	/	G	R	1	CBZ*	1(9)	N	0

AED = antiepileptic drugs; CBZ = carbamazepine; DPH = diphenylhydantoin; F = female; Follow up = seizure burden in one year after study inclusion; F = frontal; FS = focal symptomatic; G = generalized; IGE = idiopathic generalized epilepsy; L = left; LAM = lamotrigine; LEV = levetiracetam; M = male; MCA = middle cerebral artery; N = index; number of index seizures; NHS3 score = National Hospital Seizure Severity 3 score; N = normal; O = often (1/month); PFS = probable focal symptomatic; PREG = pregabalin; R = right, R = rare(1/year); Type/NHS3 = number of types/National Hospital Seizure Severity 3 score; T = temporal; TPM = topiramate; V = very often (> 1/month); VA = valproate

$p < 0.05$ ); concentrations of TNF- $\alpha$  were higher ( $Z = -2, 18$ ;  $p < 0.05$ ) and also hsCRP but the latter did not reach significance.

Patients 7 and 15 («control hyperperfusion») had the highest median concentrations of IL-6, IL-12, TNF- $\alpha$ , IFN- $\gamma$ , IL-1Ra and hsCRP among the three groups («normoperfusion», «hyperperfu-

sion», «control hyperperfusion»). Conversely, their concentration of IL-10 was the lowest among the three groups. These differences, however, did not reach statistical significance (Table 6).

The number of seizures during the follow up year correlated significantly only with the number of antiepileptic drugs ( $\rho = 0.60$ ,  $p < 0.01$ ).

TABLE 2. EEG and syndrome

EEG and syndrome		N (%)
Frequency	R	8 (27)
	O	11 (37)
	V	6 (20)
Syndrome	PFS	6 (20)
	FS	12 (40)
	IGE	6 (20)
	undetermined	6 (20)
EEG	<b>Normal</b>	4 (13)
	<b>Focal</b>	12 (40)
	<b>Generalized</b>	5 (17)
	<b>IGE</b>	6 (20)
	<b>undetermined</b>	3 (10)
Focus	RT	4(33)
	LT	5(41)
	RF	2(16)
	LF	1(8)

FS = focal symptomatic; F = frontal; IGE = idiopathic generalized epilepsy; L = left; O = often; PFS = probable focal symptomatic; R = rare; R = right; T = temporal; V = very often

Patients with a *first seizure* were analysed separately. They had a lower number of seizure types, lower NHS3-1 score, lower number of index seizures and lower number of antiepileptic drugs; but differences were not statistically significant.

TABLE 3. MRI perfusion parameters after index seizures (grey matter)

	N	M	Me	SD
<b>N</b> BF	25	101,7	93	40,8
<b>N</b> BV	25	8	7	3,9
<b>N</b> MTT	25	6,9	6,9	2,9
<b>N</b> TTP	25	13,9	13,8	2,3
<b>P</b> BF	5	265,8**	234	56,3
<b>P</b> BV	5	25,5**	27,6	4,7
<b>P</b> MTT	5	5,7	5	1,2
<b>P</b> TTP	5	14,5	13,5	4,5

BF = blood flow; BV = blood volume; M = mean; Me = median; MTT = mean transit time; N = »negative« (»normoperfusion«); P = »positive« patients (»hyperperfusion«); SD = standard deviation; TTP = time to peak; \*\* statistically significant difference (p < 0,001)

## Discussion

Elevated cytokine levels have been demonstrated after epileptic seizures in brain tissue<sup>10,11</sup> and in peripheral blood.<sup>13-15</sup> Similarly, our study found an abnormal blood level of IL-6 in 14 out of 29 patients 8–24 hours after index seizure(s).

Alapirtti *et al.*<sup>32</sup> found an increased level of IL-6 only in the group of patients with temporal lobe epilepsy, whereas in patients with extra-temporal foci cytokine the level remained unchanged. This was not the case in our patient group, in which only four (out of 30) subjects had temporal lobe epilepsy and the level of IL-6 was elevated in 14/29 patients. However, it has previously been reported that patients with active epilepsy, regardless of epilepsy type, duration or frequency of seizures, have elevated levels of IL-6.<sup>33</sup> Lehtimäki *et al.*<sup>14</sup> found an increased level of IL-6 in all but two patients with partial seizures, with the greatest magnitude in patients who suffered repeated tonic-clonic seizures; but since patients with alcohol withdrawal and tumour provoked seizures were included in their study group, the interpretation of their results is difficult.

In another study (in the emergency setting), proinflammatory cytokines were elevated postictally in half of the subjects; however, patients with brain infections, cerebral venous thrombosis and brain tumour were included<sup>34</sup>; such patients were excluded in our study.

We found a highly significant correlation not only between proinflammatory cytokines IL-6 and IL-12; TNF- $\alpha$  and hsCRP but also between proinflammatory cytokines (TNF- $\alpha$  and IFN- $\gamma$ ) and IL-1Ra. This is consistent with the hypothesis of the inflammatory damage mitigation role of IL-1Ra.<sup>35</sup>

In contrast to previous reports<sup>10,14,36,37</sup>, we found an inverse correlation between the number of index seizures and the level of proinflammatory cytokines IL-10, TNF- $\alpha$  and IFN- $\gamma$ . We suggest that this may be a consequence of the design of our study; we tested a single cytokine level and not cytokine level dynamics. Two patients with idiopathic generalized epilepsy had the highest reported number of index seizures in our group. Since we relied solely on eyewitness observational data, we did not measure the auras, exact number of index seizures or their duration, which may be a crucial factor in measurement of the postictal cytokine level. Our study was performed at emergency settings where video-EEG was not available.

MRI "normo-" and "hyperperfusion" groups of patients demonstrated different concentrations

TABLE 4. Blood cytokine concentration after index seizure(s)

	N	M	Me	SD	Ref.	No (%) above/below reference
IL-4(pg/ml)	29*	0.9	0.6	1.3	≤ 1	7(24)
IL-6 (pg/ml)	29*	5.7	3.7	5.1	≤ 3.9	15(52)
IL-10 (pg/ml)	29*	42.8	11.4	10.2	≤ 10.9	16(55)
IL-12 (pg/ml)	29*	66	58.5	40.3	≤ 204	29(100)
TNFα (pg/ml)	29*	8.5	7.6	4	4.7–12.4	1(3)
IFN-γ (pg/ml)	29*	27.5	15	54.4	≤ 1.2	7(24)
IL-1Ra (pg/ml)	29*	1302.7	676	1797	168–744	14(48)
Hs CRP (mg/l)	29*	2.9	1.1	4,5	<3	29(100)

M = mean; Me = median; N = number; Ref = reference values; SD = standard deviation; \*patient 3 is not included

TABLE 5. Median concentrations of cytokines (pg/ml) and hs CRP(mg/l) in three subgroups of patients (see text for details)

	IL-4	IL-6	IL-10	IL-12	TNF-α	IFN-γ	IL-1Ra	hsCRP
Normoperfusion	0,6	4,3	11	60,1	7,3	15,1	677	0,9
Hyperperfusion	0,3*	1,5	63,7	44,5	9,7*	14	407*	1,3
Control hyperperfusion <sup>1</sup>	0,5	6,0	5,1	110,4	10,6	27,7	868	1,6

\* Statistically significant difference ( $p < 0.05$ )

TABLE 6. Basic and control concentrations of cytokines (pg/ml) in patients with hyperperfusion after index seizure(s)

Patient	IL-4	IL-6	IL-10	IL-12	TNF-α	IFN-γ	IL-1Ra	hsCRP
2 (1 <sup>st</sup> )	0,08	<2	116	54,5	8,19	13,08	352	1,5
(control)	0,3	<2	134	99,6	8,97	9,23	822	1,7
3 (1 <sup>st</sup> )	/	/	/	/	/	/	/	/
(control)	1,77	<2	12,6	127	11,5	108	1479	0,9
7* (1 <sup>st</sup> )	0,41	2,47	10,02	24,8	11,7	44,56	676	1,9
(control)	0,35	<2	29,8	41,8	12,1	31	361	1,8
15* (1 <sup>st</sup> )	0,61	9,49	0,22	196	9,57	10,81	1060	1,4
(control)	0,00	<2	0,58	150	14,1	14,7	593	0,7
25 (1 <sup>st</sup> )	0,38	<2	11,51	34,6	11,1	14,97	462	1,1
(control)	1,01	<2	12,0	42,5	20,2	16,2	660	1,4

\* Patient demonstrated hyperperfusion also on control MRI after three months.

of cytokines IL-4 and TNF-α: patients with hyperperfusion had significantly lower concentrations of IL-4 and a higher concentration of TNF-α. The two »control hyperperfusion« patients had the highest concentrations of TNF-α and other pro-inflammatory cytokines - IL-6, IL-12, IFN-γ, IL-1Ra and hsCRP. It is tempting to speculate that »hyperperfusion« and »control hyperperfusion« patients had the highest intensity of brain inflammation but further studies will have to check this hypothesis.

There are experimental<sup>38</sup> and clinical<sup>24</sup> evidences that long lasting blood-brain barrier disruption is linked to epileptogenesis. Increased postictal blood levels of proinflammatory cytokines - as also demonstrated in our study - have been attributed to postictal blood-brain barrier affection. DSC MRI is a noninvasive surrogate biomarker of brain inflammation, capable of demonstrating endothelial dysfunction or increased blood-brain barrier permeability.<sup>35</sup> In patients with high frequency seizures, Pizzini *et al.*<sup>26</sup> demonstrated periictal hyperperfu-

**TABLE 7.** Correlation between cytokine concentrations and number of index seizures

Cytokine concentration	Pearson's coefficient of correlation $\rho$
IL-4 (pg/ml)	0,07
IL-6 (pg/ml)	0,23
IL-10 (pg/ml)	-0,37*
IL-12 (pg/ml)	0,24
TNF $\alpha$ (pg/ml)	-0,41**
IFN- $\gamma$ (pg/ml)	-0,39*
IL-1Ra (pg/ml)	-0,22
hsCRP	-0,16

\*weak correlation; \*\*moderate correlation

sion (up to 5 hours after seizure) and in patients with low frequency seizures postictal hypoperfusion (up to 15-28 hours) on both DSC MRI and non-contrast arterial spin labelling (ASL) MRI. No patient in our study had hypoperfusion. Because we chose a minimum of 8-hour postictal delay for MRI imaging (in order to avoid postictal perfusion changes that may represent ongoing epileptic activity)<sup>18,19</sup> we may have missed contralateral hypoperfusion that has proposed to be a consequence of widespread dissociation of cerebral neurovascular coupling.<sup>22</sup> We did demonstrate postictal hyperperfusion in a subgroup of patients.

MRI perfusion parameters from our group taken as a whole did not correlate with any tested variable (including seizure frequency or syndrome). Hyperperfusion on MRI - perfusion with a 12-hour delay after a 4-hour pilocarpine induced status epilepticus in rats - correlated with histological signs of neurodegeneration in certain brain areas (not seen on T2 MRI).<sup>20</sup>

Looking at the clinical data, cytokine blood levels and DSC MRI parameters, we failed to find prognostic factors for the course of disease over a one-year follow up. The main limitation of our study is the smallness and heterogeneity of our group. We propose, however, that the trends shown in our study are worth following up.

## Conclusions

In conclusion, our study revealed a subgroup of patients with brain postictal hyperperfusion and higher plasma cytokine values, indicating a probable long lasting blood-brain barrier alteration.

Larger studies are needed to validate the hypothesis that post seizure brain tissue inflammation is a crucial factor of epileptogenesis in selected patients and to determine the diagnostic and prognostic values of the various parameters studied.

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