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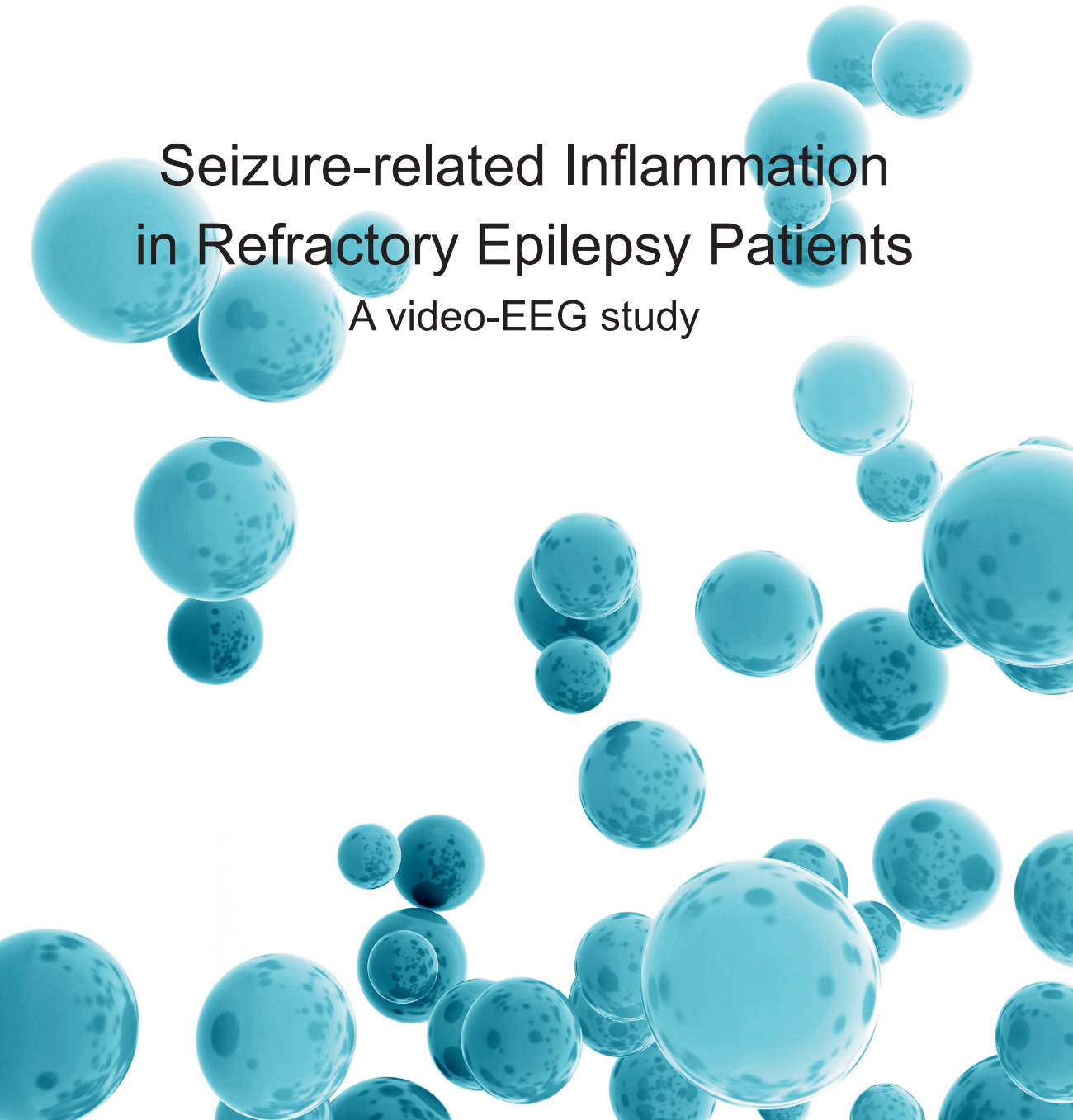
TIINA ALAPIRTTI

Seizure-related Inflammation in Refractory Epilepsy Patients

A video-EEG study



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ACADEMIC DISSERTATION
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University of Tampere, Faculty of Medicine and Life Sciences
Tampere University Hospital, Department of Neurology
Finland

Supervised by
Professor Jukka Peltola
University of Tampere
Finland

Reviewed by
Professor Laura Airas
University of Turku
Finland
Docent Eija Gaily
University of Helsinki
Finland

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To my family

'Everything should be made as simple as possible, but not simpler. '

Albert Einstein

ABSTRACT

Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures, which are transient disorders of cerebral function due to abnormal excessive or synchronous neural activity to varying extents in different anatomic brain regions. Even though there are around 30 available antiepileptic therapeutic products, a significant minority, approximately 30 % of patients suffer from refractory epilepsy with recurrent seizures. Focal refractory epilepsy with temporal lobe origin has been widely studied. During the last two decades, increasing interest in experimental and clinical studies has been focused on clarifying inflammatory seizure-related factors which are related to refractory epilepsy.

The purpose of this thesis was to investigate seizure-related inflammatory factors in refractory epilepsy patients in a well-controlled video-electroencephalographic (VEEG) environment. Seventy-eight consecutive patients with refractory focal epilepsy admitted to the VEEG monitoring unit of the Tampere University Hospital were included in the study, and of these, 51 patients experienced at least one epileptic seizure during the recording period. Plasma samples were collected for the analysis of the inflammatory markers at the beginning of the four-day recordings, on the subsequent mornings until the patient exhibited a seizure, and at 3, 6, 12 and 24 hours after the first verified seizure.

Cytokines are proteins that are involved in inter-cellular communication; they are involved in immune responses as well as regulating cell growth, tissue homeostasis and repair. In the first pilot study with 20 patients, interleukin (IL)-6 levels increased and peaked at 6 h postictally only in temporal lobe epilepsy (TLE). In experimental studies, IL-6 and IL-1β are thought to be proconvulsive, whereas interleukin-1 receptor antagonist (IL-1Ra) has shown anticonvulsive effects. Analyses of cytokines in the blood can provide information about seizure-related inflammation. Postictal plasma levels of IL-1Ra and IL-1β did not significantly differ from baseline samples, but IL-1Ra showed a decreasing trend during 12-24 h postictally.

In the final cytokine study with 49 patients IL-6 levels were increased at all time points between 3 h and 24 h. The levels of IL-6 were significantly higher at the 3 h

and 6 h time points after focal to bilateral tonic-clonic seizures (TCS) in comparison to patients with focal aware or impaired awareness seizures. The plasma level of IL-6 was significantly elevated in patients with TLE in comparison with those having extratemporal lobe epilepsy (XLE). The following characteristics were associated with an increase in IL-6 concentrations during 24 h after the index seizure; 1) a duration of the index seizure longer than 100 seconds, 2) a low baseline IL-6 level and 3) fewer than 10 seizures/month in patients with TLE were associated with an increase in IL-6 concentrations during 24 h after the IS.

In the third part of the study with 31 epilepsy patients the baseline serum levels of C-reactive protein (CRP) were significantly higher in patients with refractory epilepsy compared to controls. All five patients with elevated levels of baseline CRP had TLE vs. none in the XLE group. Seizure type during the recording was associated with a CRP increase from baseline to maximum level after the seizure. In tonic-clonic seizures CRP increased, but this did not occur in patients with focally restricted seizures. Epilepsy type was not associated with any difference in the CRP levels after the seizure.

In the fourth part of the study, the concentrations of cell-free DNA (cf-DNA) were measured from 51 epilepsy patients. Cf-DNA in plasma is released from apoptotic and necrotic cells and has displayed inflammatory properties. The baseline concentrations of cf-DNA were dependent on epilepsy type. The mean baseline concentration of cf-DNA was lower in patients with XLE as compared to control subjects. There was no significant difference in the concentration of cf-DNA between patients with TLE and control subjects. The maximum concentration of cf-DNA after baseline measurement was lower in patients with the duration of epilepsy $\geq$ 18 years compared to those with the duration of epilepsy < 18 years. The maximum concentration of cf-DNA after the seizure was higher in patients with body mass index (BMI) $\geq$ 25 compared to those with BMI < 25.

The present thesis provides evidence that in patients with human refractory epilepsy, immunological changes are related to the seizure. TLE differs from many other epilepsies in the immunological responses occurring after acute seizure. This is the first time that it has been demonstrated that only in TLE a low baseline IL-6 level and a smaller amount of seizures ($\leq$10/month) during the last year associated with an increase in IL-6 after the single seizure. If the levels of IL-6 were elevated or there had been many more seizures (>10/month) during the last year, a single seizure exerted no effect on IL-6 levels in TLE. More severe seizure types and longer seizures evoke a greater inflammatory response with higher IL-6 and CRP

levels after the seizure. Cf-DNA may be involved in refractory epilepsy; the levels after seizure are lower, if epilepsy has lasted longer. In conclusion, the immune system seems to be involved in the long-term changes such as cell loss or damage to brain networks in patients with refractory epilepsy. Inflammatory responses may either confer protection or increase the severity of the injury.

TIIVISTELMÄ (ABSTRACT IN FINNISH)

Epilepsialla tarkoitetaan taipumusta saada ilman altistavaa tekijää poikkeavan purkauksellisen aivosähkötoiminnan seurauksena ilmeneviä ohimeneviä aivotoiminnan häiriöitä eli epilepsiakohtauksia. Vaikka epilepsialääkkeitä on jo lähes 30 valmistetta, 30 prosentilla epilepsia on vaikeahoitoinen, eli lääkityksestä huolimatta kohtauksettomuutta ei ole saavutettu. Paikallisalkuinen kohtaus alkaa usein ohimolohkon alueelta, ja vaikeahoitoista ohimolohkoepilepsiaa on tutkittu runsaasti. Viimeisen 20 vuoden aikana on kiinnostuttu lisääntyvästi epilepsian vaikeahoitoisuuteen liittyvistä tulehduksellisista tekijöistä, joita on osoitettu liittyvän kohtauksiin kokeellisissa ja kliinisissä tutkimuksissa.

Tämän tutkimuksen tarkoituksena oli selvittää erilaisiin kohtauksiin liittyviä tulehdusreaktiota ja niihin vaikuttavia tekijöitä luotettavasti analysoidussa kohtauksessa neljän vuorokauden video-EEG -tutkimuksen aikana vaikeahoitoisilla epilepsiapotilailla. Tutkimukseen osallistui 78 potilasta, joista 51 sai yhden tai useampia epilepsiakohtauksia rekisteröinnin aikana. Heiltä otettiin verinäyte tulehdusmarkkerien analysointia varten rekisteröintiin tulopäivänä, sekä seuraavina aamuina, kunnes he saivat ensimmäisen epilepsiakohtauksen sekä 3, 6, 12 ja 24 h kuluttua kohtauksesta.

Sytokiinit ovat proteiinirakenteisia solujen viestinnän välittäjäaineita, jotka tunnetaan tulehdusreaktiota säätelevistä vaikutuksistaan. Kokeellisissa malleissa interleukiini(IL)-6 ja IL-1β ovat vaikuttaneet kohtauksia lisäävästi, ja IL-1 reseptoriantagonistilla(Ra) on ollut kohtauksia estävää vaikutusta. Sytokiinien määritys verestä voi antaa tietoa potilaiden kohtauksiin liittyvästä tulehduksesta. Ensimmäisessä osatyössä määritettiin sytokiinianalyysit ensin pilottityössä ensimmäisistä 20 potilaasta, ja verrattiin kohtauksen jälkeisiä pitoisuuksia ennen kohtausta otettuihin näytteisiin kullakin potilaalla. Epilepsiapotilaista vain ohimolohkopotilaiden ryhmässä tapahtui IL-6:n nousua ollen korkeimmillaan kuuden tunnin kohdalla kohtauksesta. Sytokiinien IL-1Ra:n ja IL-1β:n pitoisuudet eivät eronneet merkittävästi lähtötilanteen pitoisuuksiin. IL-1Ra -pitoisuudessa oli laskeva trendi ohimolohkopotilailla 12 ja 24 h kohdalla.

Ensimmäistä työtä jatkettiin määrittämällä IL-6 -pitoisuudet 49 kohtauksen saaneelta potilaalta. Todettiin, että IL-6 kohosi kaikissa aikapisteissä kohtauksen

jälkeen. IL-6:n nousu oli suurempi 3 h ja 6 h paikallisesta yleistyneen kohtauksen jälkeen verrattuna paikalliseksi jääneen kohtauksen jälkeen. Ohimolohkoepilepsiassa IL-6:n maksimimuutos oli merkitsevästi korkeampi muihin epilepsiaryhmiin verrattuna. Ohimolohkoepilepsiassa kohtaus nosti IL-6:n pitoisuuksia tilanteissa, joissa kohtaus kesti yli 100 sekuntia, IL-6 pitoisuus oli matalampi ennen kohtausta ja mikäli kohtauksia oli edeltävästi vähemmän kuin 10 kuukaudessa.

Kolmannessa osatyössä 31 vaikeahoitoisen epilepsiapotilaan analyysissa todettiin lähtötilanteessa CRP:n pitoisuuden olevan merkitsevästi korkeampi kuin verrokkien CRP-pitoisuus. Kaikilla viidellä epilepsiapotilaalla, joilla CRP oli lähtötilanteessa koholla, oli ohimolohkoepilepsia, mutta ei yhdelläkään muiden aivolohkojen epilepsiaa sairastavilla. Epilepsiakohtauksen tyyppi vaikutti kohtauksen jälkeiseen nousuun siten, että yleistyneet kohtaukset nostivat CRP:n pitoisuutta, mutta paikalliseksi jäävät kohtaukset eivät. Epilepsiatyypillä ei ollut vaikutusta nousuun.

Neljännessä osatyössä 51 kohtauksen saaneesta potilaan verinäytteistä määritettiin Cell-free DNA (cf-DNA) eli tulehduksen ja solukuoleman markkerin pitoisuudet. Lähtötilanteen muista kuin ohimolohkoista alkaneissa kohtauksissa cf-DNA oli matalampi verrattuna terveiden verrokkien pitoisuuteen, ohimolohkoepilepsia ei eronnut verrokeista. Yli 18 vuotta epilepsiaa sairastaneiden cf-DNA:n pitoisuudet olivat kohtauksen jälkeen matalampia kuin alle 18 vuotta sairastaneiden. Cf-DNA:n pitoisuus oli kohtauksen jälkeen korkeampi potilailla, joilla ylipainoa kuvaava ns. body mass index (BMI) oli ≥ 25 verrattuna potilaisiin, joilla BMI oli <25.

Tehdyt tutkimukset osoittavat, että vaikeahoitoisilla epilepsiapotilailla tapahtuu kohtaukseen liittyviä tulehdusreaktioita. Ohimolohkoepilepsiassa tulehdusvasteet eroavat muista lohkoista alkavista epilepsioista yksittäisen kohtauksen jälkeen, ja uutena löydöksenä oli ohimolohkoepilepsiassa IL-6:n nousu, jos IL-6 -pitoisuus oli matala tai kohtauksia oli vähemmän edeltäneen vuoden aikana. Jos kohtauksia oli enemmän tai IL-6 -pitoisuus oli korkeampi, ei yksittäinen kohtaus nostanut IL-6 -pitoisuutta. Yleistyneet ja pidempään kestäneet kohtaukset nostavat yksittäisen kohtauksen jälkeen IL-6:n ja CRP:n pitoisuuksia. Tulokset viittaavat myös cf-DNA:n osallisuuteen vaikeahoitoisessa epilepsiassa; jos sairaus on kestänyt pidempään, reaktio yksittäisen kohtauksen jälkeen on heikompi. Voidaan todeta, että tulehdusreaktioilla voi olla merkitystä vaikeahoitoisessa epilepsiassa tapahtuviin pitkäkestoisiin muutoksiin, kuten solukatoon tai aivojen eri osien väliseen toiminnan koordinaatioon. Tulehdusreaktiot voivat suojata vaurioilta tai lisätä niitä.

TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

The dissertation is based on the following original publications:

I Alapirtti T, Rinta S, Hulkkonen J, Mäkinen R, Keränen T, Peltola J. Interleukin-6, interleukin-1 receptor antagonist and interleukin-1beta production in patients with focal epilepsy: a video-EEG study. 2009. J Neurol Sci. 280(1-2):94-97.

II Alapirtti T, Lehtimäki K, Nieminen R, Mäkinen R, Raitanen J, Moilanen E, Mäkinen J, Peltola J. 2018. The production of IL6 in acute epileptic seizure: A video-EEG study. J Neuroimmunol 316:50-55.

III Alapirtti T, Waris M, Fallah M, Soilu-Hänninen M, Mäkinen R, Kharazmi E, Peltola J. 2012. C-reactive protein and seizures in focal epilepsy: A video-electroencephalographic study. Epilepsia 53(5):790-6

IV Alapirtti T, Jylhävä J, Raitanen J, Mäkinen R, Peltola J, Hurme MA, Liimatainen S. 2016. The concentration of cell-free DNA in patients in video-EEG patients is dependent on the epilepsy syndrome and duration of epilepsy. Neurol Res 38(1):45-50.

ABBREVIATIONS

AD, Alzheimer's disease
AED, antiepileptic drug
AP, accessory protein
BBB, blood brain barrier
BDNF, brain-derived neurotrophic factor
BMI, body mass index
CBZ, carbamazepine
cf-DNA, cell-free DNA
CLB, clobazam
CNS, central nervous system
CD, cortical dysplasia
COX, cyclooxygenase-2
CPS, complex partial seizure;
CRP, C-reactive protein
CSF, cerebrospinal fluid
CZP, clonazepam
DBS, deep brain stimulation
EEG, electroencephalogram
ELISA, enzyme-linked immunosorbent assay
ER, emergency room
FAS, focal aware seizure
FASE, Focal aware status epilepticus
FBTCS, focal to bilateral tonic-clonic seizure
FIAS, focal impaired awareness seizure
FLE, frontal lobe epilepsy
FS, febrile seizure
FSE, febrile status epilepticus
GABA, gamma-aminobutyric acid
GAD, glutamic acid decarboxylase
GBP, gabapentin

GTCS, generalized tonic-clonic seizure
HDL, high density lipoprotein
HIV, human immunodeficiency virus
HMGB1, high-mobility group box 1
HRT, hormone replacement therapy
HS, hippocampal sclerosis
HsCRP, high sensitive CRP
ICAM-1, intracellular adhesion molecule-1
ICU, intensive care unit
IGE, idiopathic generalized epilepsy
ILAE, international league against epilepsy
IL-1β, interleukin-1β
IL-1α, interleukin-1α
IL-1R1, interleukin-1 receptor
IL-1Ra, interleukin-1 receptor antagonist
IL-2, interleukin-2
IL-4, interleukin-4
IL-6, interleukin-6
IL-6R, interleukin-6 receptor
IL-10, interleukin-10
IL-11, interleukin-11
INF-γ, interferon-γ
iNOS, inhibited nitric oxide synthase
IS, index seizure
IVIG, intravenous immunoglobulin
LE, limbic encephalitis
LEV, levetiracetam
LNG-IUD, levonorgestrel intrauterine device
LTG, lamotrigine
MRI, magnetic resonance imaging
mRNA, messenger ribonucleic acid
NO, nitric oxide
PET, positron emission tomography
PGE2, prostaglandin E2
PHT, phenytoin
PLE, parietal lobe epilepsy

PTX3, pentraxin-related protein 3

PTZ, pentylenetetrazole

R, receptor

RE, Rasmussen's encephalitis

RTLE, refractory temporal epilepsy

SE, status epilepticus

sGp130, soluble glycoprotein 130

SIGIRR, single immunoglobulin IL-1 receptor related molecule

SPS, simple partial seizure

STAT, signal transducer and activator of transcription

SUDEP, sudden unexpected death in epilepsy

TBI, traumatic brain injury

TCS, tonic-clonic seizures

TGFβ, transforming growth factor-β

TLE, temporal lobe epilepsy

TLE+HS, TLE with hippocampal sclerosis

TLE-HS, TLE without hippocampal sclerosis

TNF-α, tumor necrosis factor-α

TPM, topiramate

VCAM-1, vascular cell adhesion molecule-1

VEEG, video-EEG

VGB, vigabatrin

VGKC, voltage gated potassium channel

VNS, vagus nerve stimulator

VPA, valproic acid

XLE, extra-temporal lobe epilepsy

1 INTRODUCTION

Epilepsy is one of the most common serious neurological disorders; the etiology of epilepsy can be divided into genetic, structural, metabolic, immunological, infectious or unknown origin. Younger patients may often have a genetic cause or some malformation of cortical development (Abdijadid et al 2015, Barkovich et al 2012) behind the epilepsy; new-onset epilepsy in the elderly is mainly caused by brain diseases, e.g. acquired causes like stroke, head trauma, or neurodegenerative diseases (Liu, Yu, Lu 2016). Despite appropriately chosen and adminstered antiepileptic medications, approximately 30 % of patients have refractory epilepsy with recurrent seizures.

The average annual cumulative incidence of epilepsy is 67.8 per 100 000 persons (Fiest et al. 2017), and one in 26 people will develop epilepsy during their lifetime (Hesdorffer et al. 2011). The incidence curve is j-shaped with a high incidence in children under 1 year of age (124/100 000) (Gaily et al. 2016), remaining at 20/100 000 in the age range 20-60 (Hesdorffer et al. 2011), then climbing even to 610/100 000 by the age 85 (Ip et al. 2017). The lifetime risk of epilepsy is 1.7 % until the age 50 but elevates to 3.4 % at the age 80. The median lifetime prevalence of epilepsy is 5.8 per 1000 in the developed countries compared to 15.4 in rural and 10.3 per 1000 in urban population in developing countries (Ngugi et al. 2010), where the higher incidence of birth trauma, traumatic brain injury (TBI) and central nervous system (CNS) infections increase the risk (Ba-Diop et al. 2014). Epilepsies of unknown etiology have the highest prevalence (Fiest et al. 2017).

Epilepsy is not only a personal burden to the individual, but it is also an economic burden due to direct costs like antiepileptic drugs (AEDs) and surgery, hospitalization for seizures, comorbidities, and also indirect costs attributable to reduced school attendance, employment and productivity losses, and caregiver burden (Allers et al. 2015). In a newly diagnosed Finnish patient with epilepsy, the long-term mortality varies from two-fold in aged 70-74 years to five-fold in 10-49 years old when compared to the general population, and both an infectious etiology of epilepsy and a higher seizure frequency increases the mortality risk (Nevalainen et al. 2012). Refractory epilepsy increases the risk for sudden

unexpected death in epilepsy (SUDEP), which is the most common in young adults aged 20-40 (Holst et al. 2013; Devinsky et al. 2016). In addition, the seizure type influences the mortality risk with tonic-clonic seizures (TCS) conferring higher risk for SUDEP than focally restricted seizures (Hesdorffer et al 2012; Ryvlin et al. 2013; Lhatoo et al. 2016). Long duration of epilepsy (>15 years) was also one risk factor for SUDEP (Hesdorffer et al 2012).

It is important to study the seizure-related factors which are related to refractory epilepsy; furthermore, it would be beneficial to unravel the relatively complicated mechanisms explaining why epilepsy may be a progressive disease with recurrent seizures, cognitive decline, brain atrophy and predispose SUDEP (Pitkänen and Sutula 2002, Vezzani et al 2011). In epilepsy there are different neuronal networks interacting within the epileptogenic structure (hippocampal or neuronal networks) and between the cortico-thalamic and basal ganglia networks (Moshé et al. 2015), and different components including inflammation may confer plasticity on these networks. The etiology of epilepsy is considered as a major risk factor for refractory epilepsy, but in some patients with drug resistant epilepsy there may be an ongoing process based on unknown mechanisms (secondary epileptogenesis) (Shorvon and Luciano 2016). There is also evidence that mechanism of epileptogenesis may be different in various etiologies (Dixit et al. 2016). There is convincing data that inflammation is involved not only in development of epilepsy, but also in the course of those epilepsies that do not respond to seizure-preventive medications (Pitkänen and Sutula 2002; Vezzani et al. 2011; Vezzani et al. 2016). The present studies were undertaken to investigate inflammatory factors comparing the situation before and 24 hours after a seizure in different refractory epilepsy types monitored under carefully supervised circumstances, with an emphasis on differences between temporal lobe epilepsy (TLE) and extratemporal lobe epilepsy (XLE).

2 REVIEW OF THE LITERATURE

2.1 Definition of the epilepsy

An epileptic seizure is defined as a transient disorder of the brain function due to abnormal excessive or synchronous neural activity to varying degrees in different anatomic brain regions (Fisher et al. 2005; Fisher et al. 2014). Seizures can affect sensory, motor, and autonomic function; consciousness; emotional state; memory; cognition; or behavior (Fisher et al. 2005).

Epilepsy is a disorder of the brain characterized by an enduring predisposition to generate epileptic seizures; it displays variable neurobiologic, cognitive, psychological, and social consequences (Fisher et al. 2005). The epilepsy syndrome is an identifiable entity with different etiologies; consists of symptoms related to epilepsy (e.g. seizure type), seizure onset and findings in the investigations (e.g. electroencephalogram). Recently epilepsy has been redefined by the International League Against Epilepsy (ILAE) to be in essence a condition where there are at least two unprovoked (or reflex) seizures occurring >24 hours part, or it can include a single unprovoked seizure if the probability of recurrence is at least 60 % over the following ten years, or if the patient has received a diagnosis of epilepsy syndrome (Fisher et al. 2014). Seizure freedom is achieved when the patient does not experience any type of seizures for 12 months or there is three times the preintervention interseizure interval, whichever is longer (Kwan et al. 2010).

Acute symptomatic seizures occur within the first week after a brain insult (e.g. stroke, TBI, infection); the risk of developing epilepsy during the next 10 years varies from 13-33 % based on the etiology of the acute seizure, although on average, it seems to be under 20 % (Beleza 2012; Hesdorffer et al. 2009). After virus encephalitis with acute seizures, there is as much as a 22-fold elevated risk for late unprovoked seizures (Misra, Tan, Kalita 2008). Patients with late unprovoked seizures, which occur often months or years after the insult, are diagnosed as having acquired epilepsy (Vezzani et al. 2016).

Epilepsy is resolved in an individual who has an age-dependent epilepsy syndrome, but is now past the applicable age or in a patient who has remained

seizure-free for the last 10 years or with no AED for the last five years (Fisher et al. 2014).

2.2 Classification of epilepsies

2.2.1 Epilepsy types and syndromes

Epilepsies can be classified according to the age, etiology, seizure type, electroencephalogram (EEG) abnormality, neuroimaging findings and concomitant diseases. In 1981 and 1989 the Commission on Classification and Terminology ILAE developed a widespread classification of seizures and epilepsy (Anonymous 1981); they were divided based on partial and generalized onset, simple and complex partial seizures and various specific generalized seizure types and epilepsies. Since then, there have been proposals that the classification should be revised (Berg et al. 2010; Berg and Scheffer 2011; Engel and International League Against Epilepsy (ILAE) 2001). The new proposal has emphasized that genetic, structural-metabolic and unknown etiology should replace the term idiopathic (presumed genetic), symptomatic (secondary to a known disorder of the brain) and cryptogenic (probable symptomatic) background of epilepsy, and to simplify all partial onset seizures under one term of "focal seizures".

In 2017, ILAE updated the classification of epilepsies for clinical diagnosis based on three levels of diagnosis: seizure type (focal, generalized or unknown onset), epilepsy type (focal, generalized, combined generalized and focal, unknown) and epilepsy syndrome (Scheffer et al. 2017). At the first level, the seizure type, an EEG finding may increase the accuracy of the diagnosis. The second level, the epilepsy type, is based on a diagnosis made according to the 2014 definition (Fisher et al. 2014). Several epilepsy types have multiple types of seizures. Ictal or interictal EEG recordings and brain magnetic resonance imaging (MRI) findings help to distinguish between the focal and generalized epilepsy types. The third level, an epilepsy syndrome, is possible to diagnose; if specific seizure types, EEG, and the imaging findings occur, and it may display a typical age at onset and remission, seizure triggers, diurnal variations, even prognosis, and mental or cognitive comorbidities.

2.2.2 Etiologies of epilepsies

The new classification of epilepsies includes the etiology at each level of diagnosis. In 2010, ILAE characterized epilepsies based on the etiologies behind the epilepsy into genetic, structural/metabolic, unknown cause, (Berg et al. 2010), and further in 2017, according to pathogenesis into six categories: structural, genetic, infectious, metabolic, immune and unknown (Scheffer et al. 2017). These groups may have an influence on the treatment of epilepsy, and they replace the earlier terms of idiopathic, symptomatic and cryptogenic epilepsy.

Structural etiology may be acquired if the patient has a brain tumor, stroke, TBI, and infection. In mesial TLE, hippocampal sclerosis (HS) is a common neuroimaging finding. If there is structural etiology, then surgery may be needed. The etiology can be multiple e.g. both structural and genetic as in tuberosis sclerosis caused by mutation in the genes TSC1 and TSC2 may produce a structural lesion in the brain (Scheffer et al. 2017). There are many known genetic mutations in which seizures are a major symptom, but in most cases, the genetic etiology is unknown. The genetic epilepsy is not always inherited as there may be a new mutation, which can be inherited by the patient's children by variable penetrance (Claes et al. 2001; Weckhuysen et al. 2012). Infectious or postinfectious etiology is the most common worldwide type, e.g. encephalitis evoked by different viruses, bacteria or parasites etiologies (e.g. herpes simplex virus, HIV, congenital infections such as Zika virus) (Vezzani et al. 2016). Cerebral folate deficiency is an example of a metabolic or genetic etiology of epilepsy, and epilepsy with prominent myoclonic seizures is a common feature of several mitochondrial disorders.

Autoimmune epilepsies are immunologically (antibody) mediated disorders in which seizures are the dominant clinical symptom with neural autoantibodies and possibly inflammatory findings in cerebrospinal fluid (CSF) or MRI (Spatola and Dalmau 2017), but autoimmune epilepsies may also be structural with hippocampal sclerosis detected in MRI (Bauer et al. 2017, Fang et al. 2017). An unknown etiology of epilepsy means that the cause of the epilepsy is still unknown, although it may be found in the EEG that the seizures may originate e.g. from the frontal lobe.

2.3 Classification of seizures

In 2017, ILAE published a new classification of seizure types (Figure 1) in order to focus on either focal onset or generalized seizure onset which has been based on 1981 classification (Fisher 2017; Fisher et al. 2017a; Fisher et al. 2017b), and also extended in 2010, and also classify seizures with unknown onset (Berg et al. 2010;

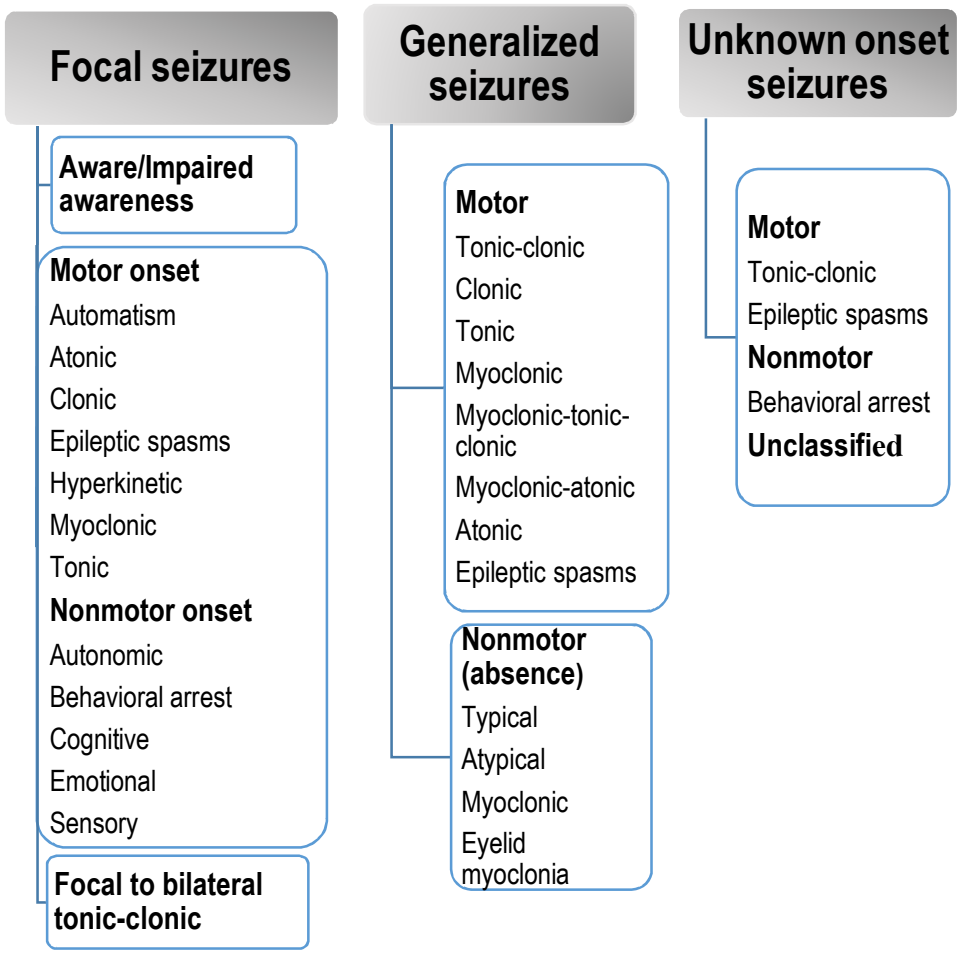


Figure 1. The ILAE 2017 operational classification of seizure types, modified from Fischer et al 2017a.

Berg and Scheffer 2011; Engel and International League Against Epilepsy (ILAE) 2001). The new classification of seizures is the basis for definition of epilepsy types and epilepsy syndromes, and etiology of epilepsy is also taken into account. There have been major changes in many terms: 1) all former "partial" onset seizures are simplified to be called "focal seizures"; 2) awareness is used to classify focal seizures to focal aware (earlier simple partial), focal impaired awareness (earlier complex partial); 3) focal to bilateral TCS replaces the former term of secondarily generalized seizure; 4) atonic, clonic, epileptic spasms, myoclonic, and tonic seizures can be classified with either a focal or generalized onset, 5) new generalized seizure types are absence with eyelid myoclonia, myoclonic absence, myoclonic-atonic, myoclonic-tonic-clonic; 6) seizures with an unknown onset may have features to still to be classified (Fisher et al. 2017a; Fisher et al. 2017b). Seizures are now divided into focal, generalized or unknown onset. The classification of seizure types is an important part of the classification of epilepsy and influences which AED should be administered to the patient.

Previous classifications have been based on anatomic region of seizure onset: temporal, frontal, parietal, occipital, dienchephalic or brainstem seizures. Seizures can be evaluated also according to the anatomic origin of the seizure.

2.4 Refractory epilepsy

2.4.1 The definition of refractory epilepsy

Despite the major developments in AEDs during the past two decades (Bialer 2011), for unknown reasons, approximately 30 % of epilepsy patients continue to experience seizures even with optimal pharmacological treatment (Kwan and Brodie 2000; Mohanraj and Brodie 2006; Sillanpää et al. 1999; Schuele and Lüders 2008). There have been many concepts used to describe refractoriness towards epilepsy treatment, and drug resistant epilepsy can be defined as a situation with the basic elements: absence of response to two AEDs tolerated at reasonable doses (whether as monotherapies or in combination); a minimum frequency of seizures (e.g. one seizure per month) or the duration of maximum seizure relapse (e.g. 6–12 months) (Beleza 2009; Kwan and Brodie 2010; Mohanraj and Brodie 2006). The ILAE published the first consensus definition of drug-resistant epilepsy; it was defined as failure to achieve sustained seizure freedom by adequate trial of two

tolerated and appropriately chosen and used AED schedules (whether as monotherapies or in combination) (Kwan and Brodie 2010; Kwan et al. 2010).

The risk for refractoriness after the failure of two AEDs has been similar between AEDs acting through different mechanisms (Kwan and Brodie 2000; Mohanraj and Brodie 2006). HS is found in 60-70 % of patients with refractory TLE, and 70 % of patients with mesial TLE suffer intractable epilepsy (He et al. 2016; Schmidt and Löscher 2005).

2.4.2 Definition of epileptogenesis

Epileptogenesis is defined as the development and extension of brain tissue capable of generating spontaneous seizures, resulting in the development of an epileptic condition and/or progression of the epilepsy after it is established (Pitkänen and Engel 2014; Terrone et al. 2016). Epileptogenesis can be caused by congenital, genetic or acquired events. The epileptogenesis in the focal epilepsy is the phenomenon of neurobiological changes which are initiated after the trigger events such as traumatic brain injury, stroke or infectious disease of the brain, or for other reasons like genetic or inflammatory factors; after a latency period, they lead to increased excitability and spontaneous epileptic seizures and the diagnosis of epilepsy (Alyu and Dikmen 2016; Pitkänen and Sutula 2002). The immune system is involved in the epileptogenesis; in particular the development of focal epilepsy in mesial temporal lobe includes complex neurobiological changes not only in neuronal networks but also at the molecular level; apoptosis, plasticity changes in neural axons and dendrites, gliosis and changes in receptors modifying neuronal activity (Figure 2). The role of inflammation may vary in various etiologies of epilepsies. In childhood epilepsy with an etiology of cortical dysplasia (CD), there are several proposed non-inflammatory mechanisms involved in the epileptogenesis in the different subtypes; e.g. in CD type I, immature and hypertrophic pyramidal neurons may lead to hyperexcitable interneuronal activity, whereas in CD type II the dysplastic neurons may provoke an ictal discharge (Abdijadid et al. 2015).

The blood brain barrier (BBB) is viewed a defence mechanism that protects the brain against many toxic molecules. The BBB is composed of endothelial cells which form a diffusion barrier, based on the presence of tight junctions that firmly

connect endothelial cells (Abbott et al. 2010). A breakdown of the BBB has been implicated in both the consequence of seizures and in the progression to epilepsy.

The currently available 30 AEDs suppress acute seizures, but they do not prevent epileptogenesis or the development of epilepsy. Disease-modifying therapies for preventing epileptogenesis are still lacking (Vezzani et al. 2015a).

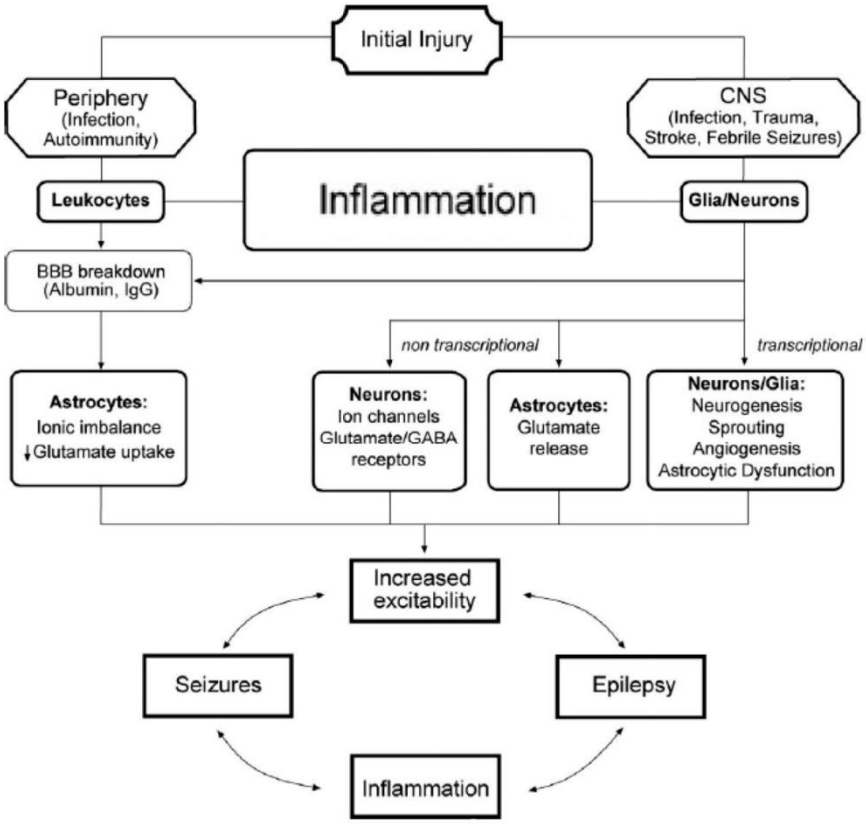


Figure 2. Pathophysiological events leading from inflammation to focal epilepsy. The brain epileptogenic injury occurring during birth or later in life in the CNS may evoke a cascade of inflammation and progressive neuronal, molecular, and network alterations leading to seizures and epilepsy. The initial epileptogenic event may originate within peripheral lymphoid tissue, such as in prolonged febrile seizures, systemic infections or autoimmune disease. Recurrent seizures can maintain inflammation or vice versa. From Vezzani et al 2014, with permission of Epilepsy Currents. BBB, blood brain barrier; CNS, central nervous system; GABA, gamma-aminobutyric acid

Recurrent seizures not only cause harm e.g. the actual seizure event can interrupt some task being done, but they also include risk for accidents, may limit education, working, and may lead to cognitive decline. Uncontrolled seizures elevate the risk of both morbidity and mortality (Nevalainen et al. 2012).

Focal epilepsy with a temporal lobe origin has been a widely studied refractory epilepsy. The neuropathological changes in patients with epilepsy were first noted nearly 200 years ago as sclerosis of the hippocampus (Thom 2014), and especially during the last decades, significant experimental and clinical evidence has been found to account for the secondary neuronal damage caused by seizures. Kainic acid-induced seizures or kindling models have revealed that seizures may alter hippocampal structures and that these may cause increased excitability with recurrent seizures and hippocampal neuronal loss. Seizure-associated neuronal damage may be reflected in volume loss, especially in the ipsilateral temporal, parietal, frontal and occipital regions, and also in contralateral regions as detected in both MRI and post-mortem studies (Blanc et al. 2011; Briellmann et al. 2002; Thom et al. 2005).

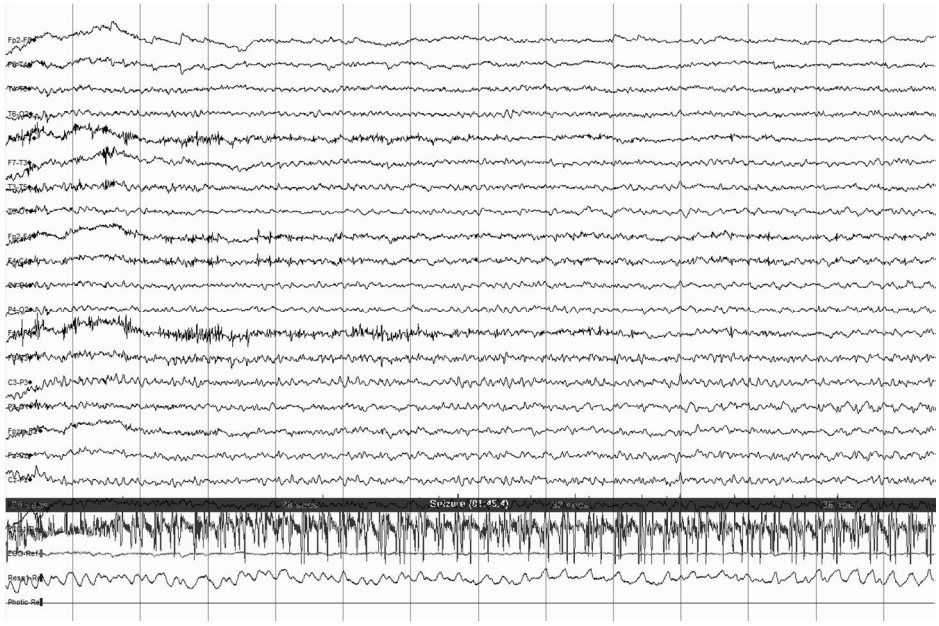
2.4.3 Treatment of refractory epilepsy

The ILAE consensus emphasises the importance of avoiding any delay in the evaluation of surgical treatment after failure of two AEDs (Kwan and Brodie 2010). Unfortunately resective surgery is not an option for all patients, or if it has failed to achieve seizure-freedom, then neurostimulation surgery, anterior thalamic deep brain stimulation (DBS) or vagus nerve stimulator (VNS), may be considered (Cukiert and Lehtimäki 2017; Nune, DeGiorgio, Heck 2015). It is also important to minimize the adverse effects of AEDs and search for new treatment options in drug-resistant patients; by continuing active treatment, seizure remission was achieved in 15 % of patients by additional AED and in 4.5 % of patients after epilepsy surgery (Callaghan et al. 2007). Appropriate and individual counselling of patients is also an extremely important part of the treatment. The inconsistent use AEDs has a greater risk for SUDEP than polytherapy (Esen Melez et al. 2017). It is also essential to exclude false refractoriness due to nonepileptic seizures or false diagnosis of epilepsy syndrome, and VEEG monitoring has proved to be an excellent tool in this process.

2.4.4 Video-EEG

Interictal EEG lasting about 30 minutes, can be normal even in patients with refractory epilepsy, or there may only be minor changes that do not help with seizure localisation. During video-EEG (VEEG) monitoring, the synchronous recording of the ictal symptoms and signs and as well as the ictal scalp EEG pattern can be used to confirm the diagnosis of a seizure disorder, to help classify seizure type(s) (Figure 3), to assess seizure frequency and identifying precipitating factors, as well as being of assistance in surgical localization (Cascino 2002; Tatum 2012). In VEEG, seizures can be more reliably registered; there may be some frontal lobe seizures that require recording with depth elctrodes. VEEG monitoring helps also to distinguish other non-epileptic disorders from epilepsy, e.g. pseudoepileptic seizures, and sometimes even cardiac arrhythmic symptoms behind the paroxysmal events. VEEG monitoring includes also the electrocardiogram, heart rate, electro-oculogram, electromyogram, and respiratory function.

A.



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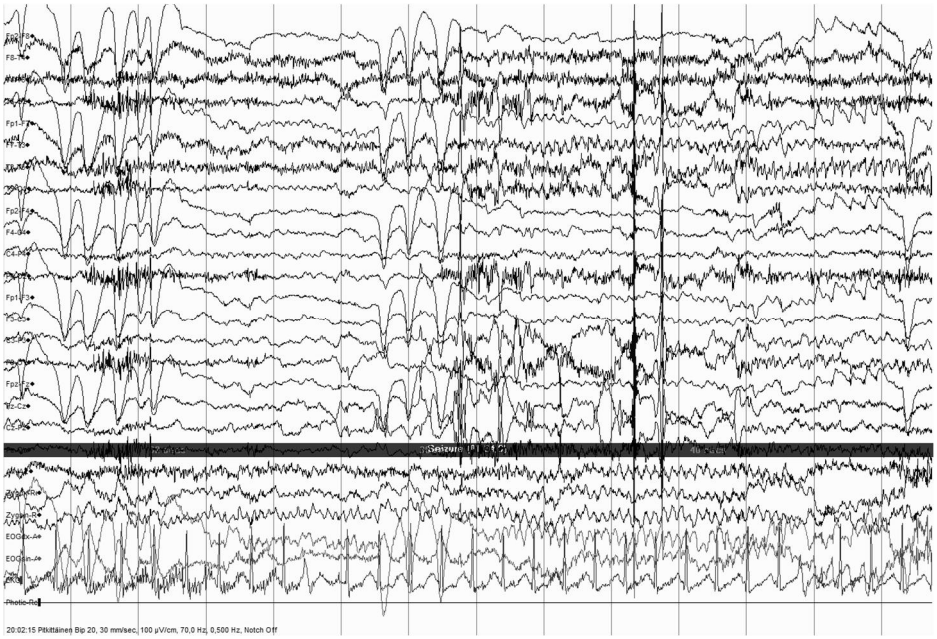

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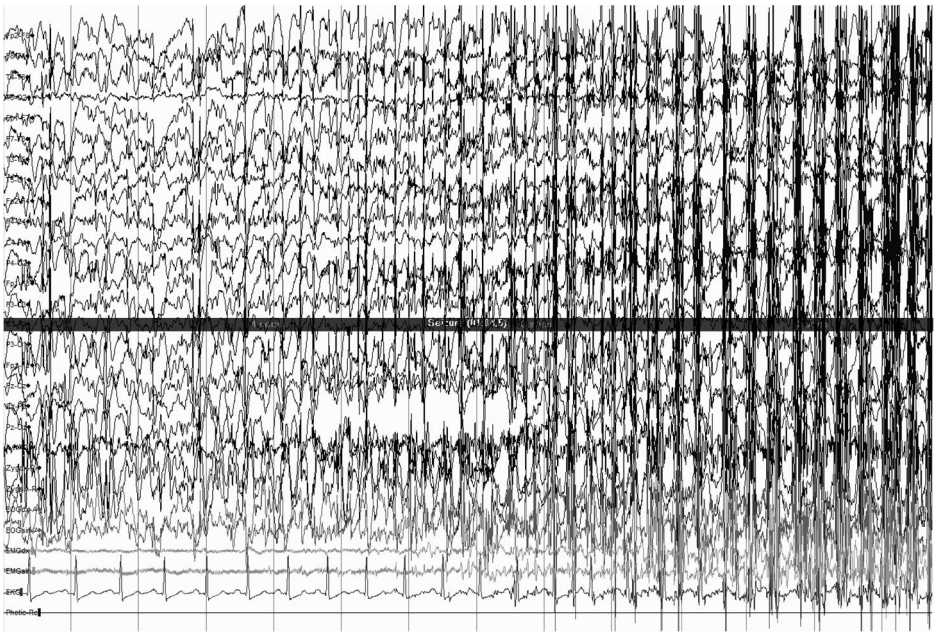

Figure 3. **Ictal epileptic activity visible in a middle of seizure in the form of discharges is shown on EEG in focal frontal seizure (A), focal temporal seizure (B) and generalized tonic-clonic seizure (C). Some muscle and movement artefacts related to motor symptoms are also present (A and C).**

2.5 Anatomical origin of focal seizures

Although the new ILAE classification is not based on anatomical brain regions, in research or in the evaluation of epilepsy surgery, there can be beneficial for classifying focal epilepsy into groups according to the anatomic origin of seizures.

2.5.1 Temporal lobe epilepsy

The temporal lobe has been widely studied in epilepsy comprising an anatomic-functional electrophysiological network that includes the amygdalo-hippocampal cortex and the entorhinal cortex close to the hypothalamus-pituitary regions (Tatum 2012). The temporal lobe is also the area most commonly damaged in infectious and autoimmune encephalitis. The hippocampal area consists of the dentate gyrus, hippocampus, and subiculum. The hippocampus is divided into cornu amnions (CA1-CA4), which have rich connections to other temporal and the extratemporal areas. This medial structure has a crucial role in memory.

Focal epilepsy in the temporal lobe, TLE, is a common epilepsy type in adults (Asadi-Pooya et al. 2017) and is categorised as having either a mesial or a lateral (neocortical) onset. In mesial TLE, the most common finding is HS, which is a combination of atrophy and astrogliosis in the amygdala, hippocampus, parahippocampal gyrus, and the enterorhinal cortex, and is viewd both a consequence and a cause of seizures in patients with TLE. HS is the most common finding in patients with epilepsy surgery and with drug-resistant TLE (Thom 2014). Both children and adults manifest TLE with HS; in one retrospective study in adults, as many as half of the patients were found to have definitive or possible limbic encephalitis (LE) based on histopathology, unilateral or bilateral hippocampal swelling or FLAIR/T2 signal increase in MRI, and antibody-findings (Bien et al. 2007). Focal CD is the most common co-existing neuropathological finding with HS in mesial TLE (Gales and Prayson 2017), and HS and focal CD can be classified to subtypes according to ILAE (Blümcke et al. 2013).

In mesial TLE, the seizures commonly start with what were previously called auras, which can be a psychic sensation (déjà vu), or a gastric sign, or a feeling of fear or an olfactory sensation (Skidmore 2016). Then typically, the dominant seizure spreads with a loss of consciousness with oral and manual automatisms, whereas a nondominant seizure may preserve awareness. ILAE has also classified HS into three types (Blumcke et al. 2013). Lateral dominant TLE seizure foci often

cause aphasia and auditory auras, and if the seizure spreads to the mesial area, the clinical symptoms are similar as described above. The seizure may also activate the motor cortex and generalize with tonic-clonic convulsion.

Multiple radiological changes have been described in patients with refractory epilepsy in mesial TLE. Progressive grey matter atrophy has been found in patients with or without HS also in seizure free patients (Alvim et al. 2016).

In temporal lobe seizures associated with loss of consciousness ictal cerebral blood flow (CBF) increases in the temporal lobe on the side of seizure onset followed by bilateral midline subcortical structures (e.g. mediodorsal thalamus), whereas blood flow decreases in fronto-parietal association cortices more prominently on the side of seizure onset (Blumenfeld et al. 2004). In the early post-ictal period, the temporal lobe is no longer activated, but there are marked increases in the blood flow of subcortical structures with known limbic connections: the bilateral medial thalamus, hypothalamus, subthalamus (midbrain–diencephalic junction), midbrain, and upper pons. In addition, the bilateral cerebellar vermis becomes activated. It has been reported that there was increased delta-range 1–2 Hz slow wave activity in the bilateral frontal and parietal neocortices during focal impaired awareness (FIAS) compared with focal aware seizures (FAS) (Englot et al. 2010).

2.5.2 Extratemporal epilepsy

2.5.2.1 Frontal lobe epilepsy

Frontal lobe epilepsy (FLE) is the second most common form of epilepsy and involves one third of the human brain (Lee and Worrel 2012). The frontal lobe can be anatomically subdivided into dorsolateral, medial and inferior orbital areas. Seizure foci have different clinical features based on their location. Dorsolateral frontal lobe can be further subdivided into primary motor cortex, premotor cortex and prefrontal cortex. Seizures originating from the primary motor cortex (Brodmann's area 4 of the precentral cyrus) and postcentral gyrus cause muscle twitches in the contralateral side of the body. The premotor cortex can be functionally divided into the secondary motor cortex (active during motor learning), the frontal eye field and Brocas's area (responsible for speech). The seizure focus in the frontal eye fields produces contralateral conjugated, saccadic eye movement and head version. The seizure focus in dorsolateral prefrontal

cortex causes problems in working memory, emotion processing and executive functions. In mesial frontal epilepsy, the seizure focus is located in the primary sensory or motor cortex in the lower limb, the supplementary sensorimotor area, the anterior cingulate cortex or the prefrontal cortex (Beleza and Pinho 2011). The seizure focus in the supplementary sensomotor area causes focal asymmetric tonic posturing, versive movements of head and eyes, speech arrest; in orbitofrontal area, they evoke complex motor automatisms, olfactory hallucinations; in the opercular area, the seizure causes salivation, swallowing, fear, facial clonic activity; in the cingulate area, they are associated with fear, vocalization emotional changes, complex motor automatisms (Bagla and Skidmore 2011; Beleza and Pinho 2011). Frontal lobe seizures are often shorter, have a very brief post-ictal state and start more often during sleep than temporal lobe seizures (Lee and Worrell 2012).

2.5.2.2 Parietal and occipital lobe epilepsy

Parietal lobe epilepsy (PLE) often involves somatosensory symptoms contralateral to the seizure foci, followed tendency by other features with a rapid ictal discharge spreading to the motor, premotor or temporo-occipital regions (Ristić et al. 2012). Seizure foci in the parietal lobe are easily mislocalized, because ictal and interital scalp EEG evaluation has shown a lower localization accuracy in PLE compared to TLE and FLE. The parietal lobe is located at the centre of multisensory integration with effective frontal and temporal neuronal network, explaining why clinical symptoms in parietal lobe seizure foci may mimic TLE or FLE. Seizure onset in the occipital area typically causes visual symptoms including hallucination, ictal blindness, blinking, nystgmus, and more complex visual scenes (Harward et al. 2017).

2.5.3 Multilobar seizures

In refractory focal epilepsy, the patient may have epileptogenic zones in more than one lobe of the brain, and often it is not possible to pinpoint where the seizure has begun. In that case, if seizure types and correlation to seizure source are not easily classified with the VEEG monitoring, sometimes depth electrode monitoring may be needed. Curative surgery may also be possible. (Jadhav and Cross 2012; Skoch et al. 2017).

2.6 Inflammation in epilepsy

There is evidence that inflammatory processes within the brain contribute to epileptogenesis and the recurrence of seizures (Bauer et al. 2017; Pitkänen and Sutula 2002; Vezzani et al. 2013). The precipitating factor, e.g. a brain insult, can activate glial cells to release proinflammatory mediators, and initiate a cascade of inflammatory processes leading to seizures and epilepsy. A disruption of the BBB may be involved in both the induction of seizures and in the progression to epilepsy, and inflammatory molecules and immune cells may pass from brain to blood and vice versa (Abbott et al. 2010). In experimental SE, seizures have been shown to evoke the induction of vascular adhesion molecules, intracellular adhesion molecule-1 (ICAM-1) and vascular cell adhesion molecule-1 (VCAM-1), and enhanced numbers of leukocytes in the brain vessels (Fabene et al. 2008). Seizures were inhibited by blockade of leukocyte-vascular adhesion e.g. linking leukocyte-vascular interactions with BBB-damage and seizures. After acute TCS in humans it was found that there were also elevated numbers of leukocytes in peripheral blood and CSF (Peltola et al. 2002). Inflammatory mediators in the brain may influence peripheral immune cells via hypothalamus- pituitary-adrenal axis (Esch and Stefano 2002).

Patients with epilepsy have also a more than a 2.5-fold increased risk of systemic autoimmune disease (Lin, Si, Xiaoyi 2016). Seizures are common in autoimmune or paraneoplastic limbic encephalitis (Toledano and Pittock 2015). Anti-inflammatory drugs have been shown to reduce seizures in selected epilepsy syndromes (Bauer et al. 2017) and immunomodulatory treatment (corticosteroids, plasma exchange, intravenous immunoglobulin) is one tool which has been utilized in refractory and super-refractory status epilepticus, especially when autoimmune encephalitis has been diagnosed or suspected (Bauer et al. 2017; Zeiler et al. 2016; Zeiler et al. 2017). Seizure control with immunomodulatory treatment has displayed restricted and unconfirmed results in observational studies (Zeiler et al. 2017). However, there is no difference in intravenous immunoglobulin (IVIG) responders between patients with antibody-mediated encephalitis or non-specific encephalitis (Zeiler et al. 2016). More specific targeted immunomodulatory therapies have been evaluated as a means of controlling seizures (Bauer et al. 2017). There are some epileptic disorders which include features of inflammation; furthermore Rasmussen's encephalitis (RE) is a rare progressive neurological disorder with refractory epilepsy; the pathogenesis of RE has been characterized by progressive neurological dysfunction and unilateral focal atrophy, inflammation in

microglia and immunological reactions (Alyu and Dikmen 2016; Varadkar et al. 2014; Venkatesan and Benavides 2015). Immunomodulatory treatment only slows, but cannot halt, the progression of RE.

There is evidence that activation of the innate and adaptive immune system and epilepsy are linked together in some human epilepsies (Vezzani et al. 2015a). Specific autoimmune diseases associated with autoantibodies have been described with seizures. In a large retrospective population-based study, the risk of epilepsy was increased among patients with autoimmune diseases; in children it was elevated by five-fold and in adults ≤ 65 years of age by four-fold compared patients without autoimmune diseases (Ong et al. 2014). Patients with the antiphospholipid syndrome had the highest risk for epilepsy i.e. a nine-fold risk elevation, patients with SLE had a seven-fold risk, in patients with type 1 diabetes or myasthenia gravis, the risk was five-fold. Slightly under every fifth TLE patient (17.5 %) with epilepsy had an autoimmune disease. In patients with both refractory and controlled epilepsies 86 % of patients with high glutamic acid decarboxylase (GAD) titers had TLE (Liimatainen et al. 2010). In a study with 105 refractory epilepsy patients, those subjects with recent seizures had a greater prevalence of IgG class anticardiolipin antibodies (29 %) compared with patients with no recent seizures (11 %) and healthy controls (13 %) (Liimatainen et al. 2009b).

There is also convincing evidence that there are inflammatory processes in the brain in neurodegenerative diseases, such as multiple sclerosis, Parkinson's disease and Alzheimer's disease (Fakhouri et al. 2015). In the CNS, cytokines may participate in repair processes of damaged tissue; however, overproduction of cytokines may lead to cell death (Kempuraj et al. 2017). Mast cells and α-synuclein are assumed to represent the link between neurons and microglia, and the release of signal molecules e.g. cytokines, may represent a bridge between BBB breakdown and neuroinflammation (Kempuraj et al. 2017). Future research should be aimed at elucidating the specific immunological mechanisms of each disease since this could possibly be beneficial in the development of specific therapies.

2.6.1 Cytokines in epilepsy

It is over 50 years ago that scientists first identified novel proteins, now called cytokines, that mediate communication between cells; they modulate immune responses, regulating cell growth and tissue homeostasis and repair (Spulber and Schultzberg 2010). Today, almost 200 cytokines have been discovered, and they are present in the circulation at picomolar concentrations. The cytokine network

influences the CNS under physiological conditions, and there is an interaction between brain and periphery via the hypothalamic-pituitary-adrenal axis (Irwin and Cole 2011).

Cytokines are involved both in the innate and adaptive immune system, and they can exert a systemic influence, e.g. fever, or they can have an effect on the same cells from which they were produced or they can act on other neighbouring cells by binding to specific receptors (Dinarello 2000). In the CNS cytokines are released as an innate immune response from the activation of astrocytes, neurons and microglia. Cytokines have multiple features in different situations; they can synergize or antagonize each other or the target cell by influencing the expression of cytokine receptors and thus they can initiate a cascade-type response (Benveniste 1992). The immune responses depend on the differentiation of T helper (Th) cells, i.e. either proinflammatory Th1 and Th17 cells or anti-inflammatory Th2 cells (Sims and Smith 2010) (Figure 4). Inflammatory cytokines are also released from the central nervous system in several diseases, such as ischemic stroke (Zhao et al. 2017), nervous tissue trauma (McKee and Lukens 2016), and Alzheimer's disease (Wang et al. 2015). The term "cytokine storm" has been used to describe hyperinflammation in sepsis or fatal influenza (Gerlach 2016).

2.6.1.1 Interleukin-1 family of cytokines

Interleukin(IL)-1 family is the largest family of interleukins; IL-1 has been the most extensively studied highly pro-inflammatory cytokine (Dinarello 1996; Dinarello 2009). IL-1 is secreted by antigen presenting cells, mainly macrophages and dendritic cells. The biological activity of IL-1 is mediated by its receptors IL-1R1 and IL-1R2 (Vasilyev, Silkov, Sennikov 2015). Initially, the IL-1 family of cytokines included two isoforms IL-1α and IL-1β which are able to affect nearly every cell type in the body, but nowadays there are at least eleven members of the IL-1 the receptor family known capable of interacting with IL-1 receptor proteins (Kwak et al. 2016; Spulber and Schultzberg 2010). IL-1 receptor antagonist (IL-1Ra) is a specific antagonist for IL-1β and IL-1α receptor, and for example, by blocking the proconvulsive cytokine, IL-1β, IL-1Ra has demonstrated anticonvulsant effects (Figure 5) (Vezzani et al. 2000; Vezzani, Balosso, Ravizza 2008) An increase in the IL-1β levels induces the production of other proinflammatory cytokines IL-6 and IL-8, but also IL-1Ra (Gallentine et al. 2017). A low ratio of IL-1Ra/IL-1β increases the likelihood to seizures; in human febrile

status epilepticus (FSE), the disrupted balance toward higher IL-1β and lower IL-1Ra values decreases the seizure threshold.

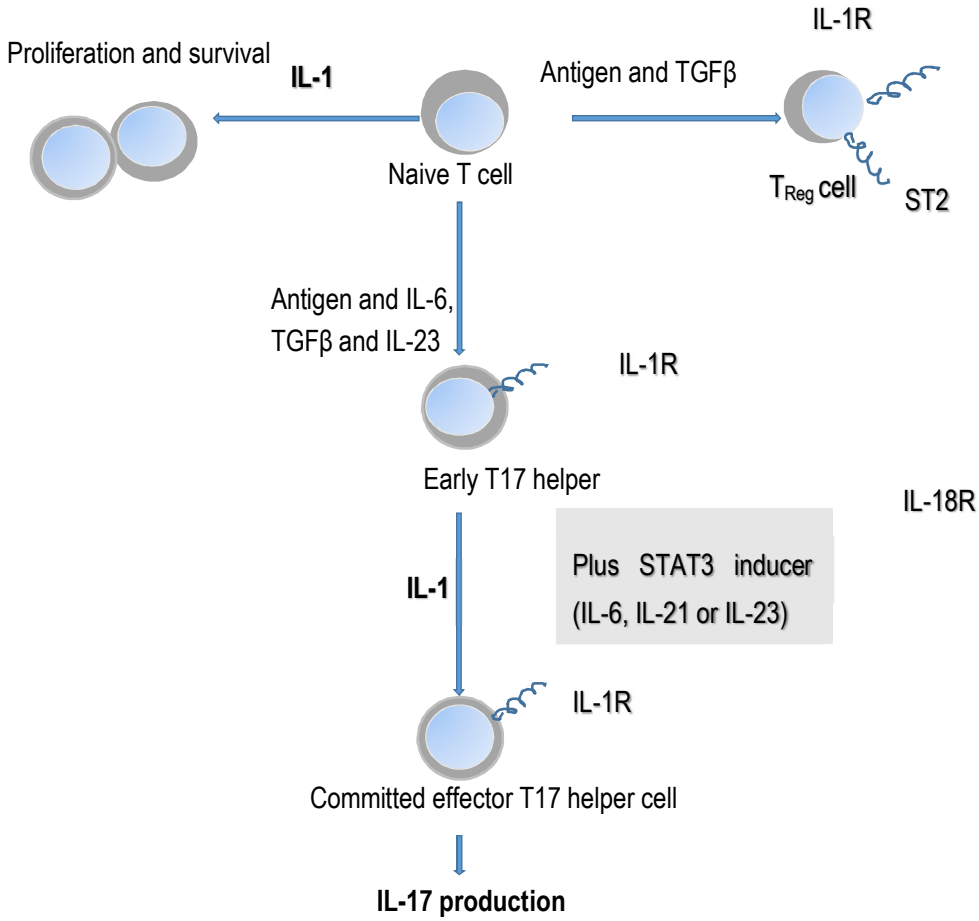

Figure 4. The effect of interleukin(IL)-1 family members on T17 helper cells, IL-1 helps proliferation of naive T-cells and development of the T17 helper cell by co-operating with signal transcuder and activation of transcription 3(STAT3)- inducing cytokines. R, receptor; TGFβ, transforming growth factor-β. Modified and adapted from Sims and Smith 2010.

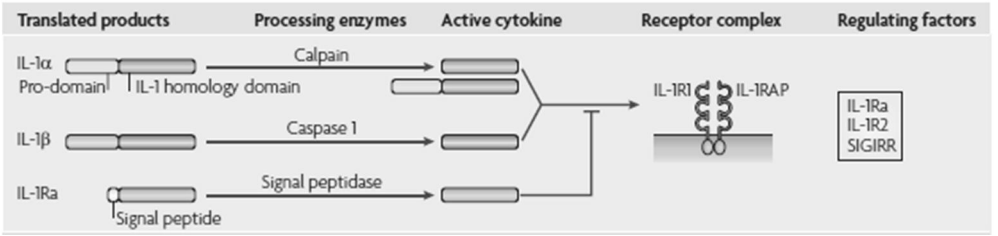

Figure 5. The biological activity of interleukin(IL)-1α and IL-1β is formed from their mRNAs with the processing enzymes, and then mediated by binding to their receptor IL-1 R1, which is expressed in the neurons in the hippocampus and other regions sensitive to seizures. There are regulating molecules that modify the final outcome. R, receptor; Ra, receptor antagonist; AP, accessory protein, SIGIRR, single immunoglobulin IL-1 receptor related molecule. Reprinted and adapted from Sims and Smith 2010 by permission of Copyright Clearance center's RightLink®.

There are some experimental findings that brain injury can activate microglia and astrocytes to release various proinflammatory mediators which may change neuronal excitability and functions of glia, thus decreasing the seizure threshold (Figure 6) (Vezzani et al. 2013). An increased production of IL-1β in glia has been shown in human TLE with HS epileptogenic tissue and in the acute phase during and shortly after SE in in experimental models (Ravizza et al 2008b) and on the other hand, by the selective blockade of IL-1β prevented kindled seizures in rats (Ravizza et al. 2008a). IL-1β has been considered to play an important role in seizure susceptibility in febrile seizures (FS): IL-1R1 deficient mice were resistant to experimental FS and exogenous IL-1β induced seizures in IL-1R1 expressing mice, but not in non-expressing animals (Dubé et al. 2005).

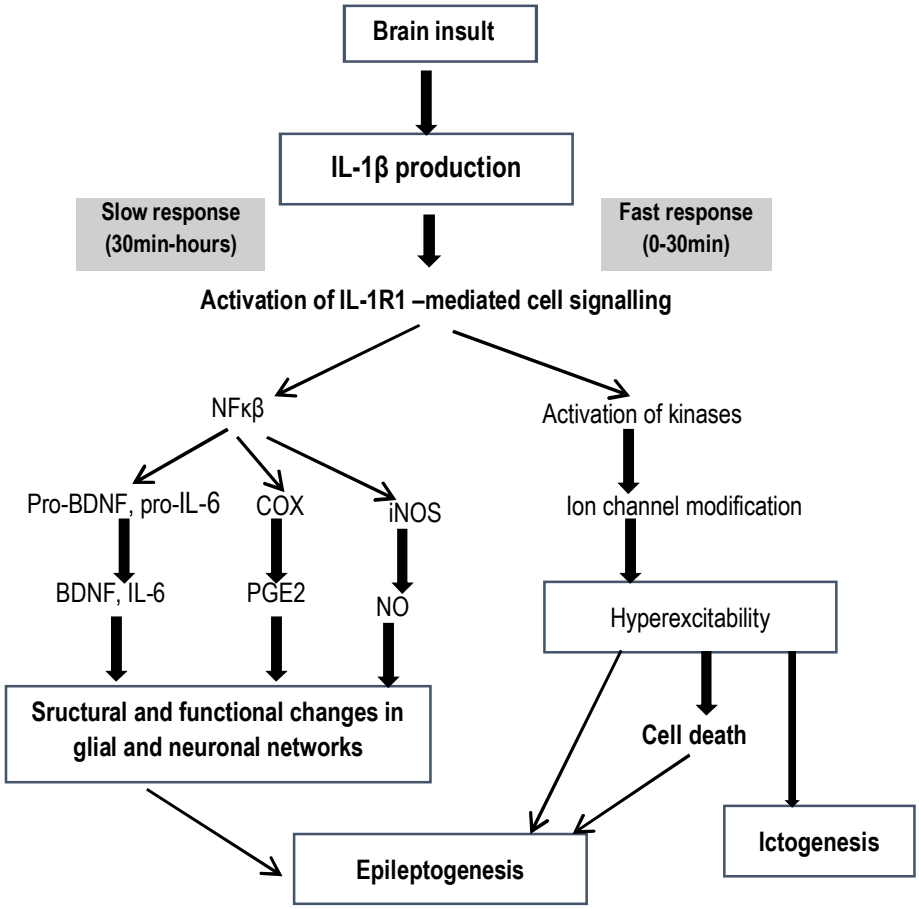

Figure 6. **After a precipitating event, the activation of interleukin(IL)-1β signalling may include the cascade with rapid effects on neuronal excitability mediated by changes in ion channels, and this may generate the first seizure (ictogenesis) or it may initiate the epileptogenetic process. Long term effects may occur e.g. via the activation $NF_{\kappa}B$; these can result in changes in glia and neural netwok and lead to epileptogenesis. Modified from Vezzani et al 2008. BDNF, brain-derived neurotrophic factor; COX, cyclooxygenase-2; IL-1R1, IL-1 receptor; iNOS, inhibited nitric oxide synthase; NO, nitric oxide; PGE2, prostaglandin E2.**

2.6.1.2 IL-6 family of cytokines

Interleukin-6 (IL-6) is considered to be proconvulsant cytokine (Kalueff et al. 2004), but also neuroprotective properties have been reported (Penkowa et al. 2001) (Figure 7). IL-6 cytokine is present in low concentrations in healthy brain (Alyu and Dikmen 2016; Lehtimäki et al. 2003). Macrophages are the main source of IL-6, but IL-6 is also produced by other cells such as endothelial cells, activated T-lymphocytes, and in CNS microglia is the main source of IL-6 (Benveniste 1992; Commins, Borish, Steinke 2010). IL-6 synthesis can be induced by other cytokines like IL-1, tumor necrosis factor-α (TNF-α) and interferon-γ (INF-γ) (Benveniste 1992). IL-6 signals through a ligand-binding IL-6 receptor α chain and the signal-transducing b-receptor glycoprotein(gp)130 which transduces also signals from several other cytokines in the IL-6 family (Wolf, Rose-John, Garbers 2014; Wolf et al. 2016).

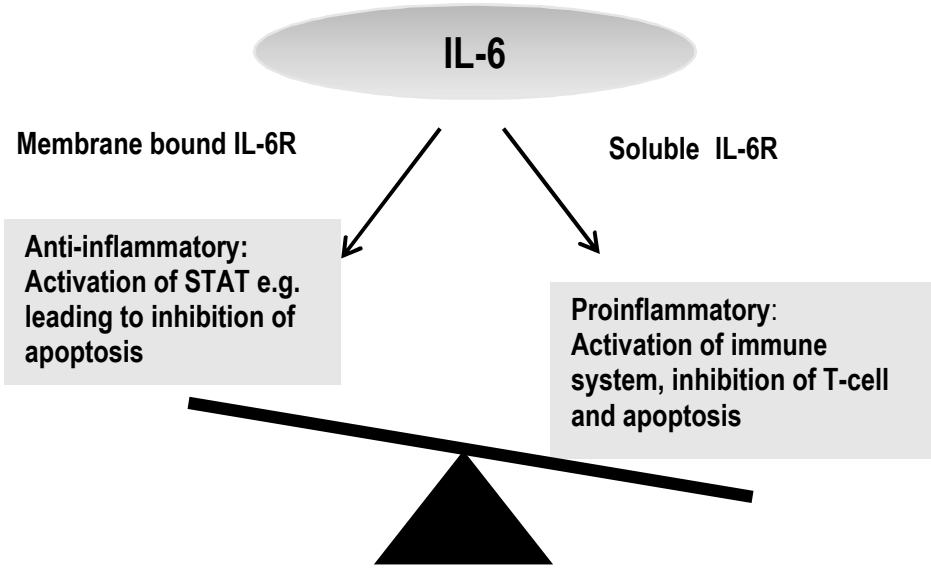

Figure 7. **Pro-and anti-inflammatory properties of interleukin(IL)-6. IL-6 stimulate target cells via membrane bound IL-6 receptor(R) with anti-inflammatory consequences, but the activity of IL-6 bound to soluble IL-6R results in inflammation. IL-6R neutralization with tocilizumab has been used to treat rheumatoid arthritis. STAT, signal transducer and activator of transcription Adapted and modified from Scheller et al. 2011.**

They activate target genes involved in differentiation, survival, apoptosis and proliferation (Commins, Borish, Steinke 2010). The soluble form of IL-6R can make a complex with circulating IL-6 and then activate Gp130 and affect distal cells (Muller-Newen et al. 1998; Rothaug et al. 2016). The family of IL-6-type cytokines contains IL-6 but also cytokines IL-11, leukaemia inhibitory factor, oncostatin M, ciliary neurotrophic factor, cardiotrophin-1 and cardiotrophin-like cytokine (Heinrich et al. 2003). IL-6 is the most prominent inducer of an acute phase pentraxin protein, CRP, and fever (Mantovani et al 2008). An increase in the serum levels of IL-6 has been linked to the total brain volume loss detected both in dementia patients (Engelhart et al. 2004) and in the general population (Jefferson et al. 2007). In the Rotterdam study which involved a population-based prospective cohort, it was observed that the elevated IL-6 levels in plasma were associated with an increased risk to develop dementia, although the levels of increased CRP associated to a lesser extent (Engelhart et al. 2004). The source of the elevated inflammatory proteins may be either peripheral stimulation or cerebral leakage through the BBB. IL-6 serum levels are elevated also in patients with an intellectual disability and active epilepsy, and IL-6 concentrations correlate with seizure severity (Lehtimäki et al. 2011).

2.6.1.3 Expression of cytokines after seizures in experimental models

Several experimental studies have demonstrated interictal and postictal (SE and single seizure) elevated levels of cytokines in the brain. After kainic acid-induced seizures, IL-6 mRNA and its signalling transducer protein Gp130 are induced in the hippocampus, amygdala and meninges, but IL-1β expression is restricted to the temporal lobe cortex and thalamus in rats (Lehtimäki et al. 2003). Cytokines and their receptor mRNA concentrations are very low before the induction of seizures. There is experimental data describing the overexpression cytokines in the brain of epileptogenic area. According to these experimental studies, IL-6 is considered as a proconvulsant mediator. Transgenic mice overexpressing IL-6 in astrocytes displayed increased sensitivity to the seizures and lethality induced by glutaminergic agonists (Samland et al. 2003). It was reported that exogenously administered IL-6 increased the severity of pentylenetetrazole (PTZ) induced seizures in rats (Kalueff et al. 2004). Kainic acid-injected IL-6 knockout mice experienced more seizures and more severe hippocampal damage and neuronal death than controls (Penkowa et al. 2001). IL-1Ra and IL-1β were induced within hours in the rodent hippocampus after seizures and the presence of IL-1β worsened seizures (Vezzani

et al. 1999; Vezzani et al. 2000; Vezzani et al. 2002; Heida et al. 2009). FSE increased hippocampal levels of IL-1β in rats, but the levels were chronically elevated only in rats experiencing spontaneous limbic seizures after FSE (Dubé et al. 2010). Pilocapine-induced status epilepticus (SE) increased the levels of IL-6 and CRP in plasma, but unlike previous studies, did not elevate the concentrations of IL-1β during epileptogenesis (Holtman et al. 2013). Both intacerebrally injected IL-1Ra and its overexpression exerted a significant anticonvulsant effect (Vezzani et al. 2000; Heida et al. 2009).

There is evidence that there are several immunologically mediated alterations in BBB integrity in epilepsy, e.g. pilocarpine induced a BBB disruption and seizures were reduced with leukocyte endothelial adhesion blockade in mice (Fabene et al. 2008). By injecting fluorescent tracers that do not enter the brain under normal circumstances, the BBB was to be found permeable in the rat limbic regions for several days in a SE rat model for TLE, and this was also observed in the latent and chronic epileptic phase (van Vliet et al. 2007). A higher seizure frequency was related to a more permeable BBB. It has been postulated that the repair of the damaged BBB could be evaluated a therapeutic target for reducing the seizures frequency (Marchi et al. 2012). BBB damage can be studied by MRI diffusion or gadolinium-enhanced techniques, vasogenic oedema is a consequence of BBB damage (Armitage et al. 2011).

2.6.1.4 Levels of cytokines after seizures in humans

There are reports of overexpression of certain cytokines in human epilepsy. In an ER setting in a trial conducted mainly in patients with acute symptomatic seizures, IL-6 levels, but not those of IL-1β or IL-1Ra, were increased in CSF in patients with TCS compared to controls (Peltola et al. 1998; Peltola et al. 2000). The plasma concentrations were lower but still reflected the elevated CSF levels indicative of intrathecal production. In a subsequent ER study IL-6 levels correlated with the seizure severity; IL-6 were clearly elevated after recurrent TCS in serum and CSF, whereas after single tonic-clonic or prolonged focal seizures (simple or complex), the levels increased to a lesser extent (Lehtimäki et al. 2004; Lehtimäki et al. 2010). CSF levels of IL-Ra increased after more severe seizures, and IL-1β decreased compared with controls (Lehtimäki et al. 2010).

The effects of single seizures in patients with chronic refractory epilepsy have been investigated in a few studies. The levels of IL-1β, IL-1Ra, IL-6 and soluble IL-6 receptors (sIL-6R and Gp130) were investigated in plasma after single seizures

during VEEG recordings in 11 patients with chronic localization-related epilepsy (Lehtimäki et al. 2007). The levels of IL-1Ra and IL-6 were elevated after seizures, whereas those of IL-1β and IL-6 cytokine receptors remained unchanged. In the second study, 25 TLE patients with FIAS or TCS levels of IL-6 increased by 51 % at 1 h postictally and remained elevated for 24 h, the levels were higher after right-sided than left-sided seizures, but there was no change in serum levels of IL-1β or TNFα (Bauer et al. 2009). In the third study investigating patients with refractory epilepsy, postictal levels of IL-6 and IL-1R were increased in all patients at 12 h, the patients were diagnosed as having TLE (n=6), XLE (n=8) and idiopathic generalized epilepsy (IGE) (n=9), but IL-1β remained unchanged (Uludag et al. 2013). In the fourth trial in which VEEG was applied to monitor twelve refractory patients, IL-6 increased postictally peaking at 12 h and remaining elevated at 24 h (Ishikawa et al. 2015). After a single febrile seizure in children, levels of IL-1β were not elevated in blood or CSF when compared to controls (Lahat et al. 1997).

IL-1β has been associated with seizure susceptibility in FS also in humans. An increased frequency of the IL-1β-511 allele 2 has been found in FS in children (Virta et al. 2002) and also in patients with TLE with HS as compared to TLE without HS, controls and patients with systemic lupus erythematosus (Kanemoto et al. 2003). After FSE, a lower ratio of IL-1Ra/IL-6 predicted mesial temporal changes in MRI, and with lower ratios of both IL-1Ra/IL-β or IL-1Ra/IL-6, the seizure threshold was lowered with an increased risk of FSE (Gallentine et al. 2017).

2.6.1.5 Levels of cytokines in chronic epilepsy

One cross-sectional study investigated 86 patients with refractory focal epilepsy; IL-6 concentrations were chronically increased in serum samples from the patients (by 11%) when compared against healthy controls (0%) (p=0.007) (Liimatainen et al. 2009a). In active focal and generalized epilepsy, interictal plasma IL-6 levels were reported to be elevated as much as 100% in comparison to controls (Nowak et al. 2011). Chronic refractory patients with epilepsy have displayed a proinflammatory cytokine profile in plasma (high IL-6, low IL1Ra and low IL1Ra/IL-1β ratio) (Hulkkonen et al. 2004).

There is evidence that the type of epilepsy influences chronic cytokine responses; increased IL-6 interictal serum levels were more common and more highly elevated in patients with TLE than in those with XLE, but concentrations of IL-1Ra were not significantly different in these patients as compared with controls

Table 1. Levels of interleukin(IL)-6 without bacterial infection or fever in patients with temporal epilepsy in humans

Authors	Design	Material	Patients (n)	Controls (n)	Results of IL-6 and other results
Hulkkonen et al. 2004	Patient vs control	Serum	Refractory temporal or frontal lobe epilepsy (10)	Healthy controls (400)	Highly pro-inflammatory cytokine profile in patients with epilepsy vs controls (high IL-6, low IL-1Ra and low IL-1Ra/IL-1 ratio
Lehtimäki et al. 2007	Patient vs control, postictal vs baseline	Serum	Refractory TLE, FLE or MLE (12)	Healthy controls (8)	The levels of IL-1Ra and IL-6 were increased after single seizures, whereas IL-1β and IL-6 cytokine receptors remained unchanged
Bauer et al. 2009	Postictal vs baseline	Serum	TLE (25)	-	After GTCS IL-6 increased by 51% immediately after the seizure ($p < 0.01$) and remained elevated for 24 h. This increase lacked in patients with HS; $n = 16$. IL-6 levels were higher after right-sided seizures as compared to left-sided seizures 24 h after the seizure (8.7 pg/mL vs. 3.4 pg/mL, $p < 0.05$). In patients taking valproate (VPA, $n = 9$), the levels of IL-1β were higher
Liimatainen et al. 2009	Patient vs control Interictal	Serum	Refractory TLE and XLE (91)	Healthy controls (63)	IL-6 was chronically increased in epilepsy patients (11%) compared with healthy controls (0%) (P = 0.007). Increased IL-6 prevalent in TLE compared to XLE (P = 0.028). The mean and the median serum levels of IL-6 were higher in TLE than in XLE (P = 0.042). IL-1Ra: no difference
Nowak et al. 2011	Patient vs control	Serum	TLE (51), TLE with HS (24), XLE (17), GE (26)	Healthy controls (36)	IL-6 was increased in all patients with epilepsy compared to controls, no difference between epilepsy types. Focal epilepsy but not GE showed a decrease in B lymphocyts

Kan et al. 2012	Patient vs control	Tissue	TLE with or without HS (32)	Autopsy patients without epilepsy or hippocampal aberrations (13)	IL-6, IL-1β or TNF-α did not show cortical or hippocampal expression. IL-10 and IL-25 were upregulated in TLE+HS patients compared to mTLE-HS and autopsy controls. CCL4 and IL-7 were upregulated in both mTLE patient groups (+ and –HS) compared to controls
Fiala et al. 2013	Patient vs control	Tissue	Refractory TLE (13)	Trauma, brain tumor, postmortem non-epilepsy (5)	Expression of IL-6 on endothelial cells in vessels. CC-chemokines and apoptotic markers (caspase-3, -8, -9) were expressed in lateral temporal cortical and hippocampal neurons of TLE patients, but not in neurons of control cases
Uludag et al. 2013	Postictal vs baseline	Serum	TLE (6), XLE (8), GE (9)	-	Increased IL-6 and IL-1Ra that peaked at 12 h postictally ($p < 0.05$). IL-1β: no difference.
Aalbers et al. 2014	Patient vs control	Tissue	TLE with HS (9)	TLE without HS (4)	IL-6, IL-1α, IL-1β, IL-1Ra, IL-8, IL-10, TNF-α, CCL2, CCL3: no difference
Strauss and Elisecich 2016	Patient vs control	Tissue	Refractory TLE (58)	Non-epileptic neurosurgical subjects with resected hippocampus, entorhinal cortex, and temporal cortex (4)	Brain IL-6 levels were highest in the temporal cortex both in epileptic and non-epileptic patients, hippocampal IL-6 was greater in non-epileptic vs. epileptic patients

AED, antiepileptic drug; hs-CRP, high-sensitivity C-reactive protein; GE, generalized epilepsy; GTCS, generalized tonic-clonic seizures; HS, hippocampus sclerosis; IL, interleukin; IL-1Ra, interleukin-1 receptor antagonist; ICAM-1, intracellular adhesion molecule-1; GE, generalized epilepsy; mTLE, mesial temporal lobe epilepsy; PTX3, plasma pentraxin 3; TLE, temporal lobe epilepsy; TNF-α, serum tumor necrosis factor α; XLE, extratemporal lobe epilepsy

(Liimatainen et al. 2009a). A recent meta-analysis also demonstrated elevated serum IL-6 levels in TLE patients (de Vries et al. 2016), suggesting that the anatomical onset of epilepsy is important in determining whether there will be any overproduction of cytokines in refractory focal epilepsy (Table 1).

In TLE patients with histopathologically-confirmed HS, tissue cytokine levels (IL-1β, IL-1α, IL-6, IL-8, IL-10, IL-1Ra, or TNF-α) were not elevated when compared with the corresponding values in patients without HS, and no difference was found between the lateralization of seizure focus (Aalbers et al. 2014).

On the other hand, inflammatory changes in microglia have been found in the seizure focus when evaluated by positron emission tomography (PET) in frontal human epilepsy, and inflammation increased after the seizure (Butler et al. 2016). In summary, it seems that inflammatory changes in epilepsy can be complex and influenced by many factors.

2.6.2 C-reactive protein (CRP)

An acute phase short pentraxin protein, CRP, is mainly produced in the liver in response to inflammatory signals, most prominently to IL-6 (Mantovani et al. 2008). One very important property of CRP is its ability to bind C1q in order to activate the classical complement cascade. Activation of complement is a crucial factor in the killing of microorganisms, i.e. CRP is involved in the reactions conferring protection from pathogenic bacteria (Marnell, Mold, Du Clos 2005).

An elevated CRP level is an established biomarker of an individual's risk of developing atherosclerotic cardiovascular disease (Ballantyne et al. 2005), but it is also found in neurodegenerative diseases such as dementia (Engelhart et al. 2004), in children with Down syndrome (DS) and later onset of dementia (Dziedzic 2006; Licastro et al. 2005; Schmidt et al. 2002). In experimental models, it has been suggested that the elevated CRP level even contributes to memory loss and to the early phase of pathogenesis of Alzheimer's disease (AD) (Lin et al. 2009). In the general population, baseline CRP levels have been higher in women (Kushner, Rzewnicki, Samols 2006), but the difference has disappeared after exclusion of women receiving hormone treatment (Hung et al. 2008). Weight-associated regulators have been found to influence the concentrations of CRP. Central adiposity has been noted most strongly to associate with CRP in women, whereas percent body fat was the strongest predictor of systemic inflammation in men and

the negative association between fitness and CRP levels was stronger in men (Valentine et al. 2009). On the other hand, plasma levels of CRP and TNF-α have not differed between lean and overweight patients nor is there any difference between men and women who gained weight due to valproic acid (VPA) treatment of epilepsy (El-Khatib et al. 2007). Large studies in the general population have revealed that CRP is higher in older people (Donato et al. 2008), and in the elderly, an elevated high sensitive CRP (hsCRP) concentration has been associated with the prediction of general mortality (Elkind et al. 2009). The Finnish Diabetes Prevention Study showed that lifestyle interventions could reduce the circulating levels of CRP and IL-6 in persons at high cardiometabolic risk (Herder et al. 2009).

2.6.2.1 C-reactive protein and epilepsy

In an ER setting in patients with acute TCS, plasma IL-6 concentrations correlated with peripheral blood leukocyte counts and CRP (Table 2) (Peltola et al. 2002). Patients with daily motor TCS exhibited higher CRP and IL-6 levels postictally in serum or plasma than patients with intermittent seizures or controls, there were no changes in pentraxin-related protein 3 (PTX3), TNF-α or IL-1β (Ishikawa et al. 2015). In patients with epilepsy surgery, CRP levels were lower in the temporal cortex than in the hippocampus or entorhinal cortex, but it was in the hippocampus where the majority of interregional associations with 25 measured inflammatory mediators were detected (Strauss and Elisevich 2016).

It has been reported that AEDs may influence the levels of inflammatory markers. In a previous study, higher CRP concentrations were associated with enzyme-inducing AEDs in patients with well controlled epilepsy, and there were induced CRP levels in those switching to lamotrigine or levetiracetam (Mintzer et al. 2009), to topiramate (Mintzer et al. 2012) or to zonisamide (Mintzer et al. 2016) lowered CRP. A recent study claimed that elevated CRP levels and increased intima media thickness at the wall of the common carotid artery could be measured in patients with epilepsy (Tan et al. 2009), i.e. there may be an increased risk of atherosclerosis either caused by the AEDs or by epilepsy itself.

Table 2. Levels of CRP without fever or bacterial infection after seizures or in patients with epilepsy in humans

Authors	Number of patients (Male/Female)	Age, mean (range)	Duration of epilepsy, mean (years) (range)	Antiepileptic drugs mentioned	Results
Peltola et al. 2002	37 patients with TCS or FBTCS <72 hour, 40 healthy controls	35.6 (16–60) vs 40.3 (16.67)	Previously undiagnosed epilepsy	Previously untreated epilepsy	Mean blood and CSF leukocyte count was increased after seizure compared to controls, correlation between plasma IL-6 and B-leucocyte count and CRP was found, CRP had positive correlation with time after seizure
Woernle et al 2013	87 patients with RTLE (73 with HS), who underwent anteromesial temporal lobe resection, 44 surgery controls with supratentorial tumor	33.7±12.9, (5–67)	23.83 (2–53)	Preoperatively: 32 with one AED, 48 with 2 AEDs, 7 with 3 AEDs. Postoperatively: 17 with 0 AED, 48 with one AED, 18 with 2 AEDs, 2 with 3 AEDs, 2 with 4 AEDs	Postoperative mean peak CRP in blood was higher in RTLE than controls in the absence of bacterial inflammation (median peak 2nd postoperative day)
Ishikawa et al 2015	12 Patients with daily seizures (DS) (7/5) , 17 intermittent seizures (IS; less than one seizure /month) (7/10) and 15 controls (7/8)	4.5 ± 2.6 (DS), 6.4 ± 2.5 (IS), 5.5 ± 3.6 (controls)	3.9± 2.5 (DS) 3.1 ± 1.9 (IS)		Interictal hs-CRP and IL-6 increased in plasma in the DS group, not increase in IS and control group. No difference in IL-1β, PTX3 and TNF-α
Talaat et al. 2015	40 patients with IGE (seizure type not described) vs 20 healthy controls	27.2 ± 7.51 vs 29.95 ± 6.01			hs-CRP and HDL higher in patients than controls, higher hs-CRP in patients on CBZ and PHT compared to VPA
Farhang et	40 patients with epilepsy	30.48± 2.03			Interictal ICAM, VCAM and CRP increased in

al. 2016	(20/20), 20 non-epileptic controls (12/8)	vs. 27.35± 3.42	patients with epilepsy cs controls, no difference in IL-1β
Strauss and Elisecich 2016	58 RTLE and 4 non-epileptic neurosurgical subjects with resected hippocampus, entorhinal cortex, and temporal cortex	39±1 vs 65±3	Brain CRP levels were lower in the temporal cortex than in hippocampus or entorhinal cortex, CRP levels were higher in 4 non-epileptic hippocampus and entorhinal cortex inpatients with emergent neurosurgery. Eotaxin, interferon-γ, IL-2, IL-4, IL-12p70, IL-17A, TNF-α, and ICAM-1 levels were highest in the hippocampus. IL-β ans IL-1α were lowest in hippocampus. IL-1β, and IL-8 levels were highest in the temporal cortex
Liguori et al. 2017	37 patients with IGE, 20 healthy controls	26.6 ± 9.49, 24.6 ± 8.16	Interictal serum C3, C4 and fibrinogen serum levels decreased in IGE, CRP increase in IGE vs controls

AED, antiepileptic drug; CBZ, carbamazepine; hs-CRP, high-sensitivity C-reactive protein; FBTCS, focal to bilateral tonic-clonic seizure HDL, high density lipoprotein; HS, hippocampus sclerosis; IL, interleukin; ICAM-1, intracellular adhesion molecule-1; IGE, idiopathic generalized epilepsy; PHT, phenytoin; PTX3, plasma pentraxin 3; RTLE, refratory temporal epilepsy; TCS, tonic-clonic seizures; TNF-α serum tumor necrosis factor α; VCAM-1, vascular cell adhesion molecule-1; VPA, valproic acid

2.6.3 Cell-free DNA

After tissue and cell damage, cells release cell-free nucleic acids into the circulation, although the exact mechanism is unknown. Experimental studies have shown that cell-free DNA (cf-DNA) in plasma is released from apoptotic and necrotic cells (Xia et al. 2016b). Cf-DNA has been shown to possess inflammatory and degenerative properties in several diseases associated with cell death and tissue damage (Butt and Swaminathan 2008). Cf-DNA was first studied in fetal medicine and oncology, but subsequently increased cf-DNA concentrations have been found in sepsis, myocardial infarction (Chang et al. 2003; Lippi, Sanchis-Gomar, Cervellin 2015), transplantation and diabetes mellitus (Butt and Swaminathan 2008; Mittra, Nair, Mishra 2012b), as well as in neurological disorders such as stroke (Rainer et al. 2003), TBI and Friedrich's ataxia (Cambello Yurkel et al. 2007; Tsai et al. 2011).

Although the origin of cf-DNA is not known, cf-DNA levels in peripheral blood correlate with the severity and outcome of TBI, stroke (Campello Yurgel et al. 2007; Tsai et al. 2011), and any disruption of the BBB may contribute to neuronal damage as well as causing cf-DNA leakage to blood. Cf-DNA levels have been claimed to predict mortality in patients with shock in intensive care unit (ICU), suggesting that cf-DNA might be used as a prognostic biomarker (Saukkonen et al. 2008; Xia et al. 2016a). On the other hand, cf-DNA has been reported to activate the innate immune system and serum levels of cf-DNA correlate with those of IL-6 and TNFα (Mittra, Nair, Mishra 2012b). In nonagenarians, plasma levels of total cf-DNA and unmethylated-DNA were higher than in young controls, and the increased levels also correlated directly with elevations in the concentrations of two inflammatory markers, CRP and IL-6, suggesting that aging and frailty are associated with immunoinflammatory activation (Jylhävä et al. 2013). When cellular damage was assessed in cancer patients, it was noted that the increase in the cf-DNA levels either caused by acute or chronic damage correlated with the degree of cellular injury (Jung, Fleischhacker, Rabien 2010). Cf-DNA has not been studied extensively in patients experiencing seizures or in those with epilepsy. In one study of patients with focal symptomatic epilepsy, 75% of patients had higher cf-DNA levels than control group (Liimatainen et al. 2013). A definite symptomatic etiology was better associated with an increased cf-DNA concentration in comparison with cf-DNA levels in cases with only a probable symptomatic etiology.

2.6.4 Putative clinical relevance of the markers of inflammation in human epilepsy

The data of pathogenesis of epileptogenesis and developing of refractory epilepsy in the experimental studies has been accompanied with human studies. Analyses of resected tissue during surgery of epilepsy patients have produced data from epilepsies with different etiologies. Different mechanisms to account for the loss of gamma-aminobutyric acid (GABA)ergic inhibition have been detected in tissue of mesial TLE and CD patients (Dixit et al. 2016), and on the other hand, an increased GABA receptor activity has been reported in severe CD but not in non-CD or mild CD (André et al. 2008). Glutamate receptor mediated excitabilitity of neurons has been found to be induced by a mutation in the gene coding for a NMDA receptor subunit in patients with idiopathic generalized epilepsy of unknown origin (Endele et al. 2010) and by upregulation of another receptors subunit i.g. GluN2B in pyramidal neurons in TLE with HS (Mathern et el. 1998). In TLE with HS, there is evidence of hippocampal neuronal and glial activation of IL-1β (Ravizza et al. 2008b). Pathophysiological levels of IL-1β in hippocampal and cortical tissue in humans may be involved in GABA-mediated neurotransmission both in TLE with or without HS, but not in non-epileptic controls (Roseti et al. 2015). IL-1β is released locally in the brain but this release is not reflected to plasma (Lehtimäki 2003).

In children, plasma samples were collected within 72 hours after FSE, and lower ratios of IL-1Ra/IL-1β, IL-1Ra/IL-6 and IL-1Ra/IL-8 were found compared to controls, but only a low ratio of IL-1Ra/IL-6 was strongly predictive of hippocampal T2 hyperintensity after FSE (Gallentine et al. 2017). The plasma IL-1Ra/IL-6 ratio may be a potential biomarker of acute hippocampal damage after FSE. Increased levels of IL-6, IL-8 and CXCL10 have also been detected in CSF in pediatric patients with refractory SE with febrile infection-related epilepsy syndrome in comparison with CFS samples from patients with other inflammatory neurological disease (Sakuma et al. 2015). In human FS, the increased levels of IL-1β suggest point to a specific role of IL-1β-mediated excitability in CNS (Yu et al. 2012).

High-mobility group box 1 (HMGB1) is a mediator of sterile neuroinflammation, and a pathologic disulfide HMGB1 isoform has progressively increased in blood before epilepsy onset in experimental models as well as in

patients with newly diagnosed epilepsy (Walker et al 2017). Patients with refractory epilepsy exhibited higher levels of total HMGB1 than in healthy controls or in those subjects with well-controlled epilepsy (Walker et al 2017). HMGB1 is known to induce the release of cytokines (IL-1β, IL-6 and TNF-α) in microglia in an experimental model (Weber et al.2015). The modulation of HMGB1 related inflammatory responses has been considered as a potential means to prevent epileptogenesis (Ravizza et al 2017.)

There are no large randomized controlled studies of inflammatory markers in human epilepsy.

3 AIMS OF THE STUDY

The purpose of this study with VEEG was to determine seizure-induced inflammatory changes in humans in a well-controlled environment. The specific aims were

1. to evaluate the production of cytokines, IL-6, IL-1Ra, IL-β, after different types of acute seizures in patients with refractory epilepsy.

2. to investigate the association of epilepsy type, e.g. TLE, and epilepsy syndrome with the inflammatory responses.

3. to search for factors during acute seizure, which might induce production of IL-6 in a larger group of patients with epilepsy.

4 MATERIALS AND METHODS

4.1 Study patients

4.1.1 Total study population

Seventy-eight consecutive patients with refractory focal epilepsy admitted to the VEEG monitoring unit of the Tampere University Hospital during November 2004 to September 2007 were included in the study. Only a few patients refused to participate in the study. Of these, 51 patients experienced epileptic seizure during recording. The study protocol was approved by the Ethics Committee of the Tampere University Hospital, and all patients provided a written informed consent. Analyses were conducted in the final cytokine and cf-DNA studies whereas the pilot cytokine and CRP studies were undertaken when enrolment was on-going, explaining the variable numbers of patients in these studies.

4.1.2 Patient populations included in the cytokine studies

In the pilot cytokine study, the first twenty consecutive patients with seizures during recording were enrolled. Patients were divided into two groups: those with temporal lobe epilepsy (TLE; n=11) and those with extratemporal lobe epilepsy (XLE; n=9) (Table 3). The mean ages of the patients in the TLE and XLE groups were 41.3 years (range 20-58) and 28.1 years (range 16-52), respectively. The mean duration of epilepsy in the TLE group was 26.4 years (range 2-52) whereas in the XLE group it was 18.6 years (range 1-52). The number of documented seizures during the last year before the monitoring was taken from patients' diaries and medical records. The mean seizure frequency per month during the previous year in the TLE group was 9.1 seizures (range 1-45), compared to 52.5 in the XLE group (range 1-200). The mean duration of the index seizure (IS) was 117 seconds in the XLE group and 69 seconds in the TLE group. The seizure burden was measured by the total duration of seizures recorded during the 24 h sampling

Table 3. Clinical characteristics of patients in pilot cytokine study

Patients with temporal lobe epilepsy								
	Epilepsy syndrome / lateralization	Age (F/M)	Duration of epilepsy years	MRI findings	Medication/ Treatment	Recorded index seizure type and lateralization	Seizures before index seizure during the previous 24 hours	Number of seizures after index seizure during next 24 hours
1*	TLE / right	36/F	17	HS	OXC 1200mg, CZP 1 mg / VNS	FIAS right	No	No
2	TLE / right	58/F	42	HS	CBZ 1400 mg, TGB 45 mg	FIAS right	No	No
3*	TLE / left	33/F	22	HS	LTG 400 mg, TPM 400 mg	FIAS left	No	8 FIAS left
4*	TLE / left	43/M	2	Normal	OXC 1500 mg	FBTCS left	No	FBTCS left
5	TLE / left	39/M	12	Normal	CBZ 1200mg, VPA 2500mg	FIAS left	No	No
6*	TLE / left	30/M	25	Hippocampal hamartoma	VPA 1500 mg, LTG 200 mg, TPM 50 mg	FIAS left	1 FIAS left	1 FIAS left
7	TLE / left	52/M	52	HS	CBZ 600 mg, LEV 2000 mg, LTG 500 mg	FIAS left	No	3 FIAS left
8	TLE / right	58/F	32	Normal	CBZ 800 mg, GBP 3600 mg, CZP 1mg	FIAS right	No	4 FIAS right
9*	TLE / left	28/M	16	HS	OXC 600mg, TPM 500 mg	FBTCS left	No	2 FBTCS left
10*	TLE / left	57/F	56	HS	CBZ 1000mg	FIAS left	1 FIAS left	2 FIAS left
11*	TLE / unknown	20 F	14	CD	OXC 1200mg, GBP 3600mg, CLB 10 mg / VNS	FAS unknown	No	2 FAS left

Patient with extratemporal lobe epilepsy								
1*	PLE / right	26/M	22	Vascular lesion	LTG 300 mg, LEV 1500 mg	FIAS right	3 FIAS right	5 FIAS right, 1 FBTCS right
2	FLE / right	22/F	3	Normal	No	FBTCS right	No	No
3	PLE / left	52/F	52	CD	TPM 400 mg, LTG 300 mg	FIAS left	No	8 FIAS left, 2 FAS left
4	FLE / left and right	16/F	1	Normal	CBZ 1500 mg, LEV 4000mg, GBP 2400 mg	FIAS right	No	1 FIAS left, 1 FBTCS right
5*	FLE / right	26/M	9	Vascular lesion	CBZ 1500 mg, LEV 4000mg, GBP 2400 mg	FAS right	1 FIAS right	5 FAS right, 1 FIAS right
6	FLE / unknown	41/F	31	Normal	PHT 250 mg, CZP 1 mg/ VNS	FIAS multifocal	No	180 FIAS seizures
7	FLE / left	31/F	31	Normal	LTG 400 mg, CBZ 1600 mg, LEV 1500 mg	FIAS left	2 FIAS left	1 FIAS left
8	FLE / left	17/F	1	CD	OXC 1200mg, TPM 300mg, LEV 3000mg	FIAS left	3 FIAS left	4 FAS left, 1 FIAS left
9	FLE / right	22/F	17	CD	OXC 1500mg, TPM 500 mg, LEV 2500 mg	FIAS right	3 FIAS right, 1 FAS right	3 FIAS right

Abbreviations: CBZ, carbamazepine; CD, cortical dysplasia; CLB, clobazam; CZP, clonazepam;; GBP, gabapentin; FAS, focal aware seizure, FBTCS, focal to bilateral tonic-clonic seizure; FIAS, focal impaired awareness seizure; FLE, frontal lobe epilepsy; HS, hippocampal sclerosis; LEV, levetiracetam; LTG, lamotrigine; OXC, oxcarbazepine; PLE, parietal lobe epilepsy; PHT, phenytoin; TLE, temporal lobe epilepsy; TGB, tiagabine; TPM, topiramate; VGB, vigabatrin; VNS, vagus nerve stimulator; VPA, valproic acid. * = IL6 level increase after the index seizure

period.

For the final cytokine study 51 patients were included for analysis of IL-6 levels before and after seizure. Samples of two patients were not available for this analysis. Based on the findings in the VEEG recordings and MRI, 23 patients had TLE, 22 patients had XLE (20 frontal lobe and two parietal lobe epilepsy) and four patients had generalized epilepsy (GE). Seven patients were receiving monotherapy, 41 had polytherapy (the mean number of medications was 2.24), and one XLE patient had no medication. In addition to AEDs, four patients were treated with vagus nerve stimulation. One female patient with right HS was monitored twice over an interval of 23 months. Clinical data of these three groups of patients are shown in Table 4. The mean number of seizures (including the IS) during the sample collection was 2.6 (range: 1-9) in the TLE group and 1.25 (range: 1-2) in the IGE group. In the XLE group, one patient had 180 and another 420 seizures during the 24 h; excluding these patients, the mean seizure number in this group was 5.4 (range: 1-18). Seven patients experienced only brief seizures during the sampling period.

4.1.3 CRP study population

In this study, the first thirty-one patients with refractory focal epilepsy were included in the second study analysing changes in their CRP levels. Based on the findings in the VEEG recordings and MRI, 15 of the patients had TLE and 16 patients had XLE (14 frontal lobe and two parietal lobe epilepsy). Six patients were on monotherapy, 24 on polytherapy (the mean number of medications 2.3), and one patient had no medication. Three patients were being treated in addition to AEDs with vagus nerve stimulator (VNS). AEDs were classified into three different categories based on their enzyme-inducing properties: non-inducing, weak-inducer, and strong enzyme-inducer. In XLE, one patient was being administered oral contraception hormone therapy, one transdermal hormone replacement therapy (HRT), and two had a levonorgestrel intrauterine device (LNG-IUD). In TLE one patient was using transdermal HRT, and one had LNG-IUD. There were three smokers in the TLE, and five in the XLE group. None of the patients were receiving statin therapy. There was no substantial difference in values of the body mass index (BMI) between the groups (TLE: mean 25.4± 3.5

Table 4. Clinical characteristics of analyzed patients in final cytokine study

	Temporal lobe epilepsy	Extratemporal lobe epilepsy	Generalized epilepsy
No. of patients	23	22	4
Male/female	12/11	11/11	0/4
Age, mean (range)	40.0 (20–58)	30.0 (16–52)	32 (27–45)
BMI, mean (±S.D.)	25.9±3.96	25.0±4.59	26.2±3.51
Mean duration of epilepsy, years (range)	23.2 (2-56)	19.6 (1-52)	18.3 (6-32)
Mean seizure frequency/month[a] (range)	8.5 (0.5-30)	59.5 (0.5-240)	1.9 (9.5-4)
Mean number of AEDs	2.17	2.50	2.25
Patient on mono-/polytherapy	4/19	2/19	1/3
MRI findings			
Normal	4	11	3
HS	13	0	Not applicable
Cortical dysplasia	3	5	Not applicable
Other	3	6	1 (vascular lesion)
PET			
Hypometabolic area	2 right, 1 left	1 right, 1 bilateral	
Normal	1		
Index seizure type			
FAS	1	3 (1 FASE)	Not applicable
FIAS	19	15	Not applicable
FBTCS	3	3	Not applicable
GTCS	-	-	4
Lateralization			
Right	4	11	Not applicable
Left	18	5	Not applicable
Right and left or unknown	1	6	Not applicable
VNS/ earlier epilepsy surgery	2/0	1/1	-/-
Duration of the IS (seconds)			
mean±S.D.	405±149	58.6±76	66±4.0
median	77	26	66
Seizure burden during sampling period (seconds±S.D.)	549±1575	1585±5661	81±31

AED, Antiepileptic drug; BMI, Body mass index =weight kg/height m^2; FAS, focal aware seizure; FASE, Focal aware status epilepticus; FBTCS, tonic-clonic seizure; FIAS, focal impaired awareness seizure; GTCS, generalized tonic-clonic seizure; HS, Hippocampal sclerosis; IS, index seizure; MRI, magnetic resonance imaging; PET, positron emission tomography; VNS, Vagus nerve stimulator,

[a] During the last year

Table 5. Clinical characteristics of patients in the CRP study

Epilepsy syndrome	No of patients	Male/ female	Age, mean (range)	Mean duration of epilepsy, years (range)	Mean seizure frequency/ month* (range)	Patients on mono- /poly- therapy	BMI kg/m^2 mean (median)	MRI findings				Index seizure type		
								Normal	HS	CD	Other	FAS	FIAS	FBTCS
TLE	15	8/7	40.3 (20-58)	24.2 (2-56)	7.4 (0.5-26)	3/12	25.4± 3.5 (26.0)	4	10	1	1	1	11	3
XLE	16	7/9	28.3 (16-52)	16.2 (1-52)	40.8 (0.5-200)	3/12	25.3±4.0 (25.4)	8	-	4	4	4	10	2

BMI, body mass index; CD, cortical dysplasia; FAS= focal aware seizure; FIAS= focal impaired awareness seizure; FBTCS: focal to bilateral tonic-clonic seizure; HS, hippocampal sclerosis; TLE, temporal lobe epilepsy; XLE, extratemporal lobe epilepsy

*During the last year

kg/m², median 26.0 kg/m²; XLE: 25.3±4.0 kg/m², median 25.4 kg/m²). Baseline characteristics of the patients are presented in Table 5.

4.1.4 Cell-free DNA study population

In the cf-DNA study, 51 patients with refractory epilepsy were analyzed to observe if changes occurred in their cf-DNA levels. Based on the findings in the VEEG recordings and MRI, 23 patients had TLE, 24 patients had XLE (21 frontal lobe, two parietal lobe and one multilobar epilepsy) and four patients had IGE. Seven patients were receiving monotherapy, 43 were administered polytherapy (the mean number of medications was 2.37), and one patient had no medication. In addition to AEDs, four patients were treated with VNS. One female patient with right HS was monitored twice over an interval of 23 months. The clinical data of these three groups of patients are shown in Table 6.

4.2 Control subjects

In the CRP study, 80 healthy volunteers served as healthy controls (66 women and 14 men; mean age 30 years, range 19-55). They have been described in more detail in a previous study (Laihia et al. 2005). In the cf-DNA study from the LASERI cohort, 141 healthy females and 109 males who were randomly selected served as control subjects (Jylhävä et al. 2012).

4.3 Methods

4.3.1 Video-EEG and classification of seizures and epilepsy

All the patients underwent continuous VEEG monitoring lasting for four days in order to acquire an electroclinical characterization of their seizures as part of the routine clinical evaluation for possible epilepsy surgery. Ictal scalp recordings were obtained using synchronous digital video and a 24-channel standard bipolar EEG. Electrodes were placed according to the International 10-20 System with additional mastoid and anterior cheek electrodes. Localization of the seizure focus was

Table 6. Clinical characteristics of patients in cf-DNA study.

	Temporal lobe epilepsy	Extratemporal lobe epilepsy	Generalized epilepsy
No. of patients	**23**	**24**	**4**
Male/female	**12/11**	**11/13**	**0/4**
Age, mean (range)	**40.0 (20-58)**	**30.0 (16-52)**	**32 (27-45)**
BMI, mean (±S.D.)	**25.9±3.96**	**25.0±4.39**	**26.2±3.51**
Mean duration of epilepsy, years (range)	**23.2 (2-56)**	**19.3 (1-52)**	**18.3 (6-32)**
Mean seizure frequency/month[a] (range)	**8.5 (0.5-30)**	**56.4 (0.5-240)**	**1.9 (9.5-4)**
Mean number of AEDs	**2.17**	**2.58**	**2.25**
Patient on mono-/polytherapy	**4/19**	**2/21**	**1/3**
MRI findings			
Normal	**4**	**12**	**3**
HS	**13**	**-**	
Cortical dysplasia	**3**	**6**	
Other	**3**	**6**	**1**
PET			
Hypometabolic area	**2 right, 1 left**	**1 right, 1 bilateral**	
Normal	**1**		
Index seizure type			
FAS	**1**	**3 (1 FASE)**	
FIAS	**19**	**17**	
FBTCS	**3**	**3**	
GTCS	**-**	**-**	**4**
VNS/earlier epilepsy surgery	**2/0**	**2/1**	**-/-**
Duration of the IS (seconds)			
mean±S.D.	**405±149**	**57.4±73**	**66±4.0**
median	**77**	**26**	**66**
Seizure burden during sampling period (seconds±S.D.)	**549±1575**	**1609±5429**	**81±31**

AED, antiepileptic drug; BMI, body mass index =weight kg/height m^2; FAS, focal aware seizure; FASE, Focal aware status epilepticus; FBTCS, focal to bilateral tonic-clonic seizure; FIAS, focal impaired awareness seizure; GTCS, generalized tonic-clonic seizure; HS, hippocampal sclerosis; IS, index seizure; MRI, magnetic resonance imaging; PET, positron emission tomography; VNS, vagus nerve stimulator.

[a] During the last year

recorded, and seizures were categorized as focal aware, focal impaired awareness, generalized tonic-clonic or focal to bilateral tonic-clonic seizures. The first unequivocally verified seizure during the VEEG monitoring was considered as the IS. All other seizures after the IS during the next 24 h were also registered.

All patients underwent a diagnostic brain MRI examination on a 1.5 (General Electric, Sigma HD, Milwaukee, Wisconsin, USA) or 3.0 (Siemens Healthcare, Magnetom Trio A Tim system 3T, Erlangen, Germany) Tesla device. Seizures and epileptic syndromes were classified according to the ILAE diagnostic criteria (Commission on Classification and Terminology of the International League Against Epilepsy, 1981) (Anonymous 1981). Anticonvulsant medications were discontinued in a stepwise fashion during the monitoring according to the evaluation of the staff epileptologist.

4.3.2 Processing of the samples

Plasma samples were collected at the beginning of the four-day recordings, on following mornings until the patient experienced seizure, and at 3, 6, 12 and 24 h after the first verified seizure (IS; index seizure). The serum samples were stored at -70 ^{o}C until analyzed. The last sample before the IS was used as the baseline sample.

4.3.3 Measurements of IL-6, IL-1Ra and IL-1β

Commercial ELISA kits were used according to the manufacturer's instructions to detect levels of IL-1Ra (Quantikine® Human IL-1Ra Immunoassay, R&D Systems, Minneapolis, MN, USA) and IL-6 (PeliKine® Compact Human IL-6, CLB, Amsterdam, The Netherlands) in pilot cytokine study.

Absorbance values were read in a Multiskan MS version 4.0 spectrophotometer at a wavelength of 450 nm. IL-1β levels were measured with a Luminex kit (High sensitivity Human cytokine Lincoplex Kit, St. Missouri, USA) using the Bio-Plex suspension array system (Bio-Rad laboratories Hercules, CA, USA). IL-1β data were collected and analysed using Bio-Plex Manager™ software 4.1 (Bio-Rad Laboratories) and a 5-parameter regression formula was used to calculate the sample concentrations from standard curve. The detection limits of the assays were 22 pg/ml for IL-1Ra, 0.4 pg/ml for IL-6, and 0.06 pg/ml for IL-1β. The intra-assay and inter-assay precisions were 6.2 % and 6.7 % for IL-1Ra, 3.11 % and 2.16

% for IL-1β, and for IL-6 the manufacturer provided an estimate for both parameters as being between 5 and 10 %. In the final cytokine study, plasma concentrations of IL-6 were determined by enzyme immunoassay (EIA) with commercial reagents (PeliPair ELISA, Sanquin, Amsterdam, The Netherlands).

4.3.4 High sensitivity measurement of CRP

High sensitivity measurement of CRP was performed in the Department of Virology, University of Turku, Finland, as described earlier (Koskinen et al. 2004). High sensitivity measurement of CRP, also referred to as hsCRP, was used to detect lower concentrations of the protein more accurately than possible with the standard test, which measures a much wider range of CRP levels but is less sensitive in the lower range.

4.3.5 Cell-free DNA measurement

The concentration of circulating cf-DNA was measured directly in serum using a Quant-iT™ DNA High-Sensitivity Assay kit and a Qubit® fluorometer (Invitrogen, Carlsbad, CA, USA) following the standard protocol according to the manufacturer's instructions. All the samples were analyzed in duplicate, and the mean of the two replicates was taken as the final value. A third sample was analyzed if the difference between the two values was more than 20%.

4.3.6 Statistical methods

In the first cytokine pilot study, Friedman analysis of variance (ANOVA) with post hoc comparisons (Wilcoxon matched pairs test) and Mann-Whitney U-test were used to compare concentrations of circulating IL-6 and IL-1Ra. Fisher´s exact test was used for comparison of dichotomous variables. Findings were considered statistically significant at P values less than 0.05. Statistical calculations were carried out using Statistica (ver. Win 5.1D, Statsoft Inc., Tulsa, OK, USA). The sample taken on the first day of the recording was used as the baseline sample.

In the final cytokine study, descriptive statistics (frequencies or means and standard deviations (SD)) were derived to summarize the patients' characteristics. Medians, means and standard error of the means (SEM) were calculated in the

comparison of differences between maximum IL-6 value after IS and the baseline concentration of IL-6. First, the difference between the IL-6 variables was calculated (maximum minus baseline concentration), and due to the positive skewed distribution, Mann-Whitney U test or Kruskal-Wallis test was applied to evaluate the difference between the maximum and baseline IL-6 concentration. Linear regression analysis was used examine the relationship between log-transformed baseline IL-6 levels and age (≤32, >32), epilepsy syndrome, TLE with or without HS, etiology, index seizure type, lateralization, AEDs, and duration of index seizure (<100, ≥100) on log-transformed baseline IL-6 levels. Each model was adjusted for age and sex excluding an examination of age (≤32, >32) which was adjusted only for sex. Geometric mean ratios from linear regression models were calculated by taking the exponential value of the coefficients. Analysis of variance (ANOVA) for repeated measures was used to investigate between- and within-groups changes in the log-transformed IL-6 level over five time points (from baseline to 3, 6, 12, and 24 hours). All analyses were conducted using Stata statistical software version 13.1 (StataCorp, College Station, Texas, USA) and IBM SPSS Statistics for Windows, version 22. In all of the statistical tests, p-values <0.05 were considered significant.

In the CRP study, due to the positive skewness in the distribution of CRP levels, log-transformation was applied in almost all of the analyses. The P value and geometric mean ratio from log-transformed linear regression analysis of CRP level (adjusted for age and sex) were calculated using Stata software, 8th version. CRP levels were also categorized into normal and elevated by cut-off of the mean CRP level plus two standard deviations in the controls (5.8 mg/l). Logistic regression was used for binomial CRP seropositivity data analysis. When logistic regression analysis was not applicable due to zero cells, Fisher's exact test was performed. Log-transformed repeated measures analysis of variance (ANOVA) was performed, using MedCalc software version 10.4.0.0, to assess the change in CRP levels from baseline to 3, 6, 12, and 24 h after the IS. Linear and quadratic trend analyses were performed to identify if there were any trends in the CRP values after the IS. Variables such as age, duration of epilepsy, duration of IS, number of AEDs etc. were used as continuous variables in the analyses when applicable. For illustration purposes and for assessing between-groups and -within-group effects, they were categorized into two groups based on their mean or median depending on their distribution.

In the cf-DNA study, a descriptive analysis was used to outline the characteristics of the participants via scatterograms, frequencies, means and

standard deviations. Due to the small sample size ordered logistic regression models (also known as proportional odds models) were used to test if there was any association between the concentration of cf-DNA at baseline (dependent variable) and the patient's age, etiology of epilepsy, duration of epilepsy, seizure frequency, or BMI (independent variables). We used the median as a cut point to divide patients' ages, duration of epilepsy, seizure frequencies, and BMI values into two groups. In order to use ordered logistic regression models continuous cf-DNA was categorized into quartiles. Each model was adjusted for age and gender, and the assumption of proportional odds (i.e. that regression coefficients would be constant across levels of the dependent variable) were tested for all models by the test of parallel lines. The difference between the baseline and maximum concentration of cf-DNA after the baseline was calculated. Mann-Whitney U-test was used to compare the distributions of these differences with respect to patients' age, duration of epilepsy, seizure frequencies and BMI values. Kruskal-Wallis test was used in assessing the in etiology of epilepsy. The significance of all tests was set at 0.05 and data analysis was performed using IBM SPSS Statistics version 19.

5 RESULTS

5.1 Cytokine production in video-EEG patients with refractory epilepsy in serum

5.1.1 Levels of IL-6, IL-1β and IL-1Ra in the pilot cytokine study

In the pilot cytokine study, the TLE group showed a significant increase in plasma IL-6 levels, peaking at 6 hours postictally (Table 7). On the contrary, there was no change between baseline and postictal IL-6 levels in the XLE patients. Postictal plasma levels of IL-1Ra and IL-1β did not significantly differ from the baseline levels in either of the patient groups. IL-1Ra showed a decreasing trend ($p=0.059$) in the TLE patients during 12 to 24 hours postictally. No correlation was found between the production of IL-6 and IL-1Ra and the etiology of epilepsy (Data not shown. Concentrations of IL-6 in the individual patients before and after the index seizure are shown in Figure 8. When only maximum concentrations of individual patients during the sampling period were considered, the mean IL-6 concentration in focal seizures were 1.44 pg/ml in the TLE group; 0.58 pg/ml in the XLE group; 6.80 pg/ml in TCS in TLE and 1.07 pg/ml in XLE.

5.1.2 Levels of IL-6 in the final cytokine study

Based on results of the pilot study, investigations into IL-6 were extended to the entire patient data. Postictal plasma levels of IL-1Ra and IL-1β had not significantly differed from the baseline levels in the pilot study, so they were not examined in this part of the work.

Table 7. Circulating concentrations of IL-6, IL-1Ra and IL-1β and IL-1Ra/ IL-1β ratio in the pilot cytokine study after the onset of seizure in temporal (n=11) and extratemporal epilepsy (n=9), values are mean ± SD.

	Epilepsy syndrome	0 hr	3 hr	6 hr	12 hr	24 hr	P(Friedman ANOVA, df=4)
IL-6 pg/ml	Temporal	0.127± 0.422	0.546± 1.46	1.89± 2.49	1.55± 2.87	0.691± 0.919	0.008[b]
	Extratemporal	0	0	0[a]	0.067± 0.200	0.411± 0.756	0.031[c]
IL-1Ra pg/ml	Temporal	490± 461	510± 443	532± 481	555± 368	425± 257	0.042[d]
	Extratemporal	313± 96.8	325± 133	331± 81.1	365± 133	334± 90.2	NS
IL-1β pg/ml	Temporal	1.75± 1.51	1.78± 1.51	1.40± 1.12	2.45± 3.67	1.71± 1.86	NS
	Extratemporal	1.58± 2.18	1.76± 2.81	1.66± 2.18	1.94± 2.46	2.06± 2.78	NS
IL-1Ra/ IL-1β	Temporal	479± 408	467± 460	704± 689	716± 994	947± 1046	NS
	Extratemporal	477± 5512	650± 518	749± 724	935± 909	659± 742	NS[e]

[a] P (Mann-Whitney U-test, Temporal vs. Extratemporal) p=0.016
[b] P (Post hoc, Wilcoxon matched pairs test, df=1) 0 hr < 6 hr, p=0.028, 3 hr < 6 hr, p=0.028, 3 hr < 12 hr, p=0.043, 6 hr > 24 hr, p=0.018
[c] P (Post hoc, Wilcoxon matched pairs test, df=1) 0, 3, 6 hr<24 hr, p=0.109
[d] P (Post hoc, Wilcoxon matched pairs test, df=1) 12 hr > 24 hr, p=0.059
[e] P (Post hoc, Wilcoxon matched pairs test, df=1 3 hr < 24 hr, p=0.043
df = degrees of freedom

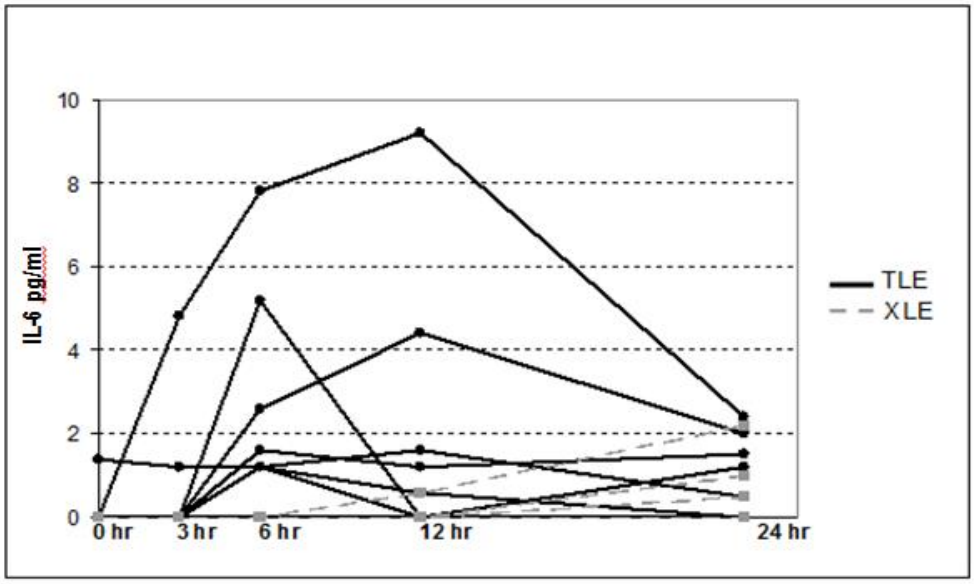

Figure 8. Concentrations of IL-6 (pg/ml) in the individual patients before (0 hour) and after the index seizure in the pilot cytokine study. TLE, temporal lobe epilepsy; XLE, extratemporal lobe epilepsy.

5.1.2.1 The baseline levels of IL-6

The baseline levels of IL-6 were not significantly different between epilepsy types (Table 8). In patients with a FAS or FIAS as the IS, the IL-6 baseline concentration was significantly higher compared to patients with TCS (focal to bilateral or generalized) as the IS (6.65 vs. 2.85 pg/ml, unadjusted model p=0.024 and sex- and age-adjusted model p=0.022). There was no effect of pre-VEEG seizure frequency, patient's age, etiology, the use of enzyme-inducing AED, duration of epilepsy, duration of IS or lateralization on the baseline IL-6 concentrations.

5.1.2.2 Postictal serum levels of IL-6

In the final cytokine study with forty-nine patients the levels of IL-6 were significantly increased at all time points between 3 h and 24 h after the IS compared to the baseline when all seizures were analyzed (p=0.032 3 h, p=0.019 6 h, p < 0,001 12 h and 24 h; Wilcoxon signed ranks test). Levels of IL-6 were significantly higher

Table 8. **IL6 level at baseline, and repeated measurements at 3, 6, 12, and 24 hours after the index seizure in the final cytokine study. The significant parameters are highlighted with bold characters.**

	Baseline IL6 (pg/l)						Measurements 0-24h	
	n	Median	Mean	SD	GMRadj	P_{LTLR}	P_{ANOVA}	$P_{Quadratic}$
Patients with epilepsy	49	4.22	5.87	7.05			**0.025** [W]	0.12
Age (mean 40, range 20–58)							0.070 [B]	
≤32 [Ref]	25	4.22	6.94	8.94	1.00		0.12 [W]	0.15
>32	24	4.27	4.76	4.22	0.79	0.42	0.077 [W]	0.55
Epilepsy type							0.52 [B]	
XLE (male/female 11/13) [Ref]	22	4.80	5.60	4.08	1.00		0.14 [W]	0.84
TLE (male/female 12/11)	23	4.33	6.76	9.43	0.98	0.95	0.14 [W]	0.30
IGE (male/female 0/4)	4	2.41	2.22	1.46	0.47	0.20	0.22 [W]	0.20
Hippocampal sclerosis (HS)							0.99 [B]	
TLE without HS [Ref]	9	4.56	9.69	14.2	1.00		0.67 [W]	0.98
TLE with HS	14	4.27	4.88	4.13	0.70	0.51	0.054 [W]	0.19
Etiology							0.81 [B]	
Unknown [Ref]	18	4.80	5.56	4.01	1.00		0.24 [W]	0.30
Hippocampal sclerosis	14	4.27	4.88	4.13	0.81	0.57	0.054 [W]	0.19
Cortical dysplasia	7	4.22	6.82	6.27	1.20	0.69	0.57 [W]	0.56
Other	10	2.20	7.16	13.4	0.65	0.31	0.27 [W]	0.47
Index seizure type							0.27 [B]	
FBTCS / GTCS [Ref]	10	2.80	2.85	2.23	1.00		0.052 [W]	0.096
Focal (FAS, FIAS, FASE)	39	4.44	6.65	7.66	2.30	**0.022**	0.058 [W]	0.52
Lateralization							0.81 [B]	
Right [Ref]	14	4.39	4.73	2.95	1.00		0.11 [W]	0.40
Left	21	4.21	6.92	9.84	0.90	0.77	0.33 [W]	0.35
Right and left or unknown	14	3.43	5.44	4.77	0.80	0.59	0.21 [W]	0.15
Using enzyme-inducing AED							0.29 [B]	
No [Ref]	15	3.33	6.12	5.82	1.00		0.27 [W]	0.17
Weak (OXC)	13	4.56	8.09	11.5	1.19	0.66	0.21 [W]	0.15
Strong (CBZ /PHN)	21	3.53	4.32	3.30	0.87	0.73	0.057 [W]	0.23
Duration of index seizure (median 1 min, range 5s-5min)							0.98 [B]	
<100s [Ref]	37	4.22	6.40	7.86	1.00		0.53 [W]	0.65
≥100s	12	3.64	4.25	3.33	0.83	0.60	**0.019** [W]	0.070
Low baseline IL6 (<5 pg/ml)							0.60 [B]	
XLE [Ref]	12	2.21	2.52	1.40	1.00		0.28 [W]	0.76
TLE	15	1.98	2.50	1.55	0.96	0.90	**0.007** [W]	**0.039**
High baseline IL6 (≥5 pg/ml)							0.79 [B]	
XLE [Ref]	10	8.91	9.30	2.94	1.00		0.35 [W]	0.74
TLE	8	10.1	14.8	12.8	1.58	0.16	0.33 [W]	0.12
≤10 seizures/month[a]							0.91 [B]	
XLE [Ref]	5	4.84	4.62	3.54	1.00		0.28 [W]	0.26
TLE	17	3.18	6.16	10.5	0.93	0.92	**0.020** [W]	0.083
>10 seizures/month[a]							0.70 [B]	
XLE [Ref]	17	4.75	5.89	4.28	1.00		0.50 [W]	0.85
TLE	6	7.35	8.47	5.95	1.81	0.21	0.51 [W]	0.34

[a],during last year, Adj GMR: Geometric mean ratio [geometric mean in a group divided by mean in the reference (Ref.) group] adjusted for age and sex, LTLR: log-transformed linear regression adjusted for age and sex, ANOVA: Log-transformed repeated measures analysis of variance (B: Test of between-groups effects, W: Test of within-groups effects); Quadratic: Quadratic trend analysis, H_0 to max: Difference between baseline IL6 value and maximum value after seizure, MWT: Mann-Whitney test or Kruskal-Wallis test if three or more groups,; AED: Antiepileptic drug; CBZ: carbamazepine, FAS: focal aware seizure; FASE: Focal aware status epilepticus; FBTCS: focal to bilateral tonic-clonic seizure; FIAS: focal impaired awareness seizure; OXC: oxcarbazepine, PHN: phenytoin, GTCS: generalized tonic-clonic seizure, SD: Standard deviation

at the 3 h (p=0.007) and 6 h (p=0.053) time points after tonic-clonic seizures including both focal onset and primary generalized seizures in comparison to FAS and FIAS (Figure 9). Postictal levels of IL-6 were higher at the 3h time point after FBTCS (P=0.019) and GS (P=0.040) when compared to FIAS. Levels of IL-6 were significantly higher at the 6h time point after focal to bilateral tonic-clonic seizures (FBTCS) and generalized tonic-clonic seizures (GTCS) compared to those after FAS at the same time; corresponding P values were 0.028 and 0.050 (Table 8).

There was a significant difference between TLE and XLE with an elevated plasma level of IL-6 in patients with TLE (Table 9). The TLE patients with frequent seizures displayed a smaller increase after single seizures compared with those with infrequent seizures (Figure 9). The higher the total seizure burden during the recording, the greater the IL-6 response.

No correlation was found between the production of IL-6 and the duration of epilepsy or BMI in any patient or any of the in seizure type subgroups. Lateralization of epilepsy exerted no influence on the absolute change of IL-6 levels, in patients with left lateralization, the relative change was higher than in patients with right lateralization (mean 1.17 vs. 0.44; median 0.28 vs. 0.17), but the difference was not statistically significant (Mann-Whitney test p= 0.47 and 0.84).

The following characteristics were associated with an increase in the IL-6 concentrations during 24 h after the IS; 1) a duration of the index seizure longer than 100 seconds (Table 9), 2) a low baseline IL-6 (Figure 10) and 3) fewer than 10 seizures/month in patients with TLE were associated with an increase in the IL-6 concentrations during 24 h after the IS (Figure 9). There was also an increasing tendency for elevated IL-6 levels between repeated measurements of IL-6 levels after the IS in older patients (p= 0.077), as well as in TLE patients with HS (p= 0.054) and in patients using strong enzyme-including AEDs (p= 0.057).

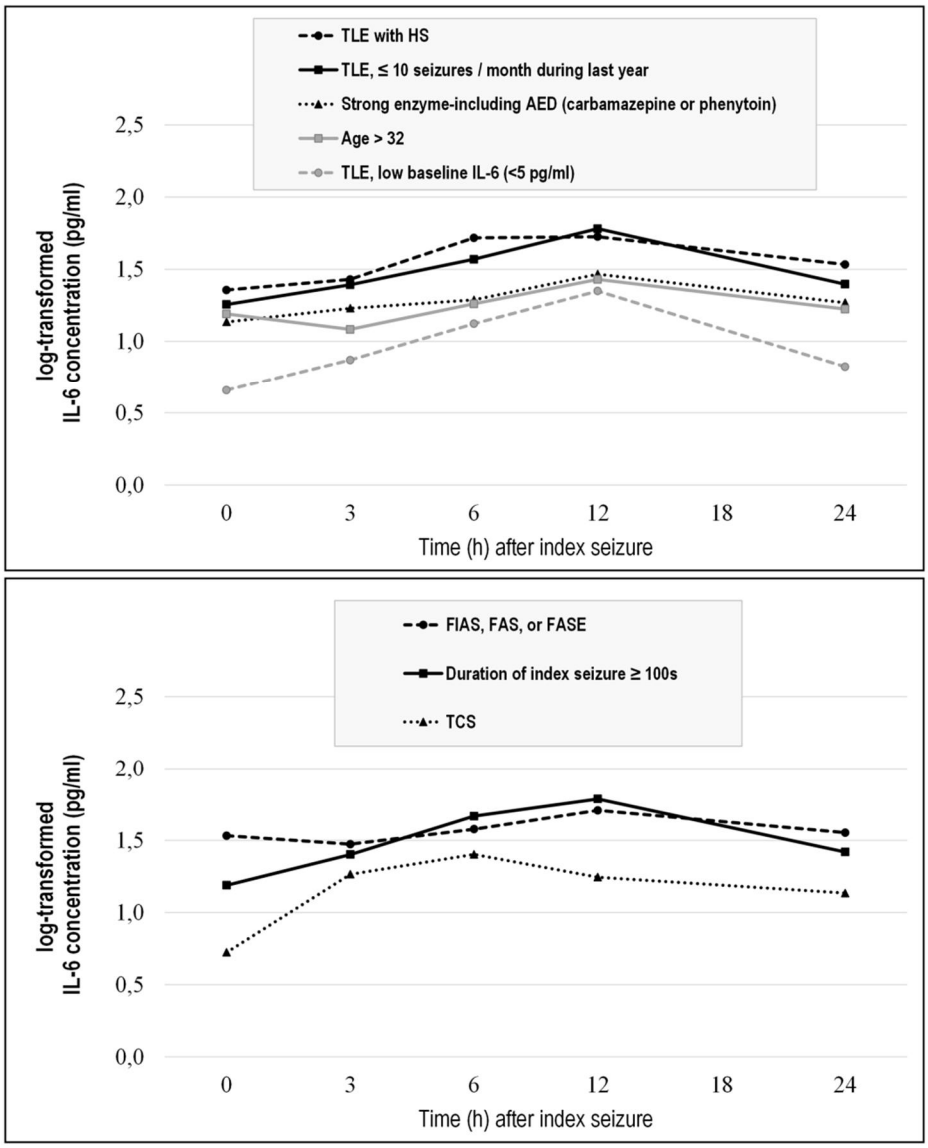

Figure 9. IL-6 levels before (0 hours) and 3, 6, 12, and 24 hours after the index seizure in the final cytokine study A. in patients with TLE with HS; in patients with TLE ≤ 10 seizures/month during last year; in patients being treted with strong enzyme-inducing AED (carbamazepine/phenytoin); in patients with age over 32 years of age; and in patients with TLE, low baseline IL-6 (< 5 pg/ml) B. in patients with FIAS, FAS, or FASE; in patients with duration of the IS ≥ 100seconds, and in patients with TCS.
AED, Antiepileptic drug; CPS, Complex partial seizure; IS, index seizure; FAS, focal aware seizure, FIAS, focal impaired awareness seizure; HS, hippocampal sclerosis; TCS, tonic-clonic seizure; TLE, temporal epilepsy

Table 9. Difference between maximum and baseline concentration of IL-6 in the final cytokine study. The significant parameters are highlighted with bold characters.

Table 10.

	n	Median	Mean	Std. error	P [1]
Patients with epilepsy	49	0.94	1.81	0.53	
Age					0.38
≤32	25	1.21	2.49	0.71	
>32	24	0.87	1.11	0.79	
Epilepsy type					**0.035**
XLE	22	0.69	0.93	0.20	
TLE	23	1.47	2.19	0.97	
Lateralization of seizure					0.41
Left	21	1.14	1.85	1.04	
Right	14	0.71	1.57	0.44	
IS type					0.32
Tonic-clonic	10	1.74	3.48	1.49	
Focally restricted	39	0.93	1.38	0.54	
Duration of epilepsy					0.93
≤18	24	0.86	2.71	0.81	
>18	24	1.10	0.91	0.70	
Seizures per month last year					**0.020**
≤10	26	1.52	3.02	0.73	
>10	23	0.61	0.44	0.69	
Duration of index seizure (sec)					0.16
≤65	25	0.81	1.02	0.83	
>65	24	1.52	2.63	0.63	
Seizure burden[a] (sec)					**0.030**
0-99	17	0.60	0.11	0.91	
100+	32	1.33	2.71	0.61	
BMI (kg/m^2)					0.78
<25	22	1.11	1.37	0.26	
≥25	27	0.93	2.17	0.95	

XLE = extra-temporal lobe epilepsy; TLE = temporal lobe epilepsy; IGE = idiopathic generalized epilepsy; IS = index seizure; BMI = body mass index.

[1] Mann-Whitney test

[a] all seizures during the sampling period (including IS)

5.1.3 The association of baseline IL-6 concentration on the seizure induced production in TLE and XLE in the final cytokine study

If the baseline level of IL-6 was low (under 5pg/ml), the IS induced a significantly greater elevation in both absolute and relative IL-6 concentrations in patients with TLE but not in those with XLE (median relative change 1.10 vs. 0.28, p =0.006 Independent Samples Median Test; median absolute change 3.01 vs. 0.52; Mann-Whitney U test p=0.004) (Figures 9 and 10). In terms of the maximum increase from the baseline IL-6 level (i.e. the highest IL-6 level assayed in the four measurements after the IS minus the baseline level), Mann-Whitney U test also confirmed the difference between TLE and XLE patients with a low baseline IL-6 concentration (<5 pg/ml) (p=0.003) (Table 10). The trend analysis also showed a significant quadratic trend (downward U-shape) for patients with TLE and low baseline IL-6 levels (Table 10, p=0.039). This trend was not evident if the TLE or the XLE patients exhibited a high baseline IL-6 concentration (median absolute change 0.59 vs. 0.86, NS; median relative change 0.07 vs. 0.52; NS). In XLE, the baseline level had no influence on IL-6 concentrations after the IS.

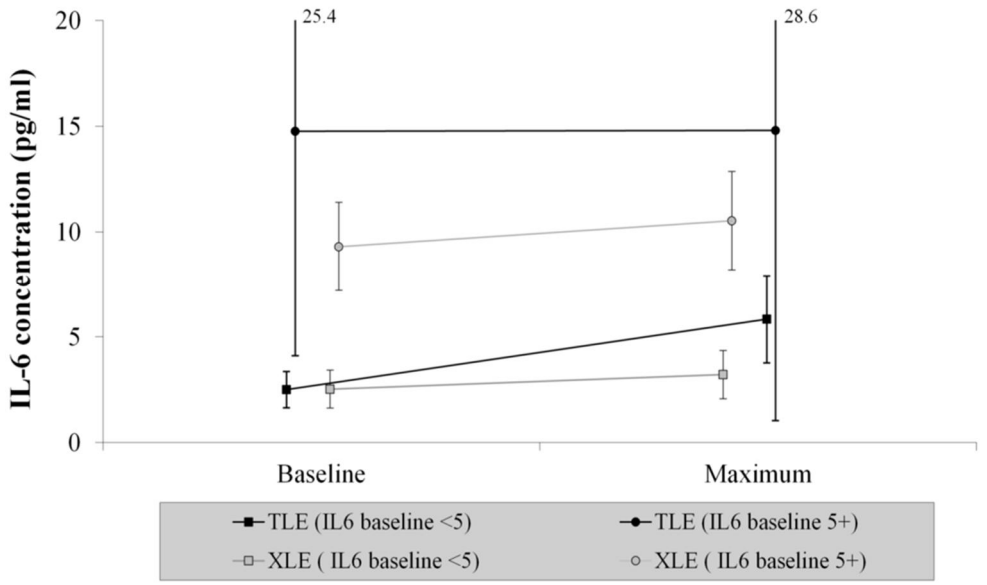

Figure 10. The effect of baseline IL-6 concentration on the seizure induced maximum production in TLE and XLE in the final cytokine study. XLE = extratemporal lobe epilepsy; TLE = temporal lobe epilepsy

Table 11. The effect of baseline IL-6 concentration on the seizure induced production in temporal epilepsy (TLE) and extratemporal epilepsy (XLE).

	TLE			XLE		
	N	Mean (sem)	Med (min / max)	N	Mean (sem)	Med (min / max)
Mean relative change in patients with low baseline IL-6 (<5)	15	2.06 (0.61)	1.10 (-0.01 / 8.62)	12	0.39 (0.15)	0.28 (-0.28 / 1.58)
Mean relative change in patients with high baseline IL-6 (5+)	8	-0.01 (0.10)	0.07 (-0.73 / 0.23)	10	0.14 (0.05)	0.10 (0.03 / 0.60)
Mean absolute change in patients with low baseline IL-6 (<5)	15	3.34 (0.76)	3.01 (-0.01 / 9.74)	12	0.68 (0.19)	0.52 (-0.59 / 1.90)
Mean absolute change in patients with high baseline IL-6 (5+)	8	0.04 (2.30)	0.59 (-13.7 / 10.2)	10	1.23 (0.37)	0.86 (0.22 / 4.19)

5.2 Plasma levels of CRP in refractory epilepsy patients before and after the seizure

The baseline serum levels of CRP (CRP-0h) were significantly higher in patients with refractory epilepsy compared to controls (3.5 vs. 0.7 mg/ml, $p<0.001$). All patients with epilepsy had a CRP-0h level higher than 0.1 mg/l (Figure 11). The mean CRP-0h was higher in TLE group than in XLE but not significantly (sex- and age-adjusted geometric mean ratio: 1.5, 95% CI: 0.6-3.9). When CRP-0h levels were converted into a binomial variable, all five patients with elevated CRP-0h [>5.8 mg/l (mean+2SD in controls)] had TLE and only one control had an elevated CRP-0h level (TLE vs. none in XLE, $p=0.018$; cases vs. control $p=0.006$; TLE vs. control, $p<0.001$; TLE+HS vs. TLE-HS, $p=0.600$).

In patients whose epilepsy was being treated with strong enzyme-inductors (carbamazepine or phenytoin) the mean CRP-0h levels were twice as high as in patients receiving non-inducing drugs, but this did not reach statistical significance ($p=0.084$). Higher CRP-0h levels were associated with a lower number of AED ($p<0.001$**).** Higher CRP-0h levels were also detected in those subjects with an older age at diagnosis (or shorter duration; $p=0.058$) (Table 11).

The baseline CRP level did not show any significant association with sex, etiology, seizures frequency during the year before index video-EEG, index seizure type, or duration of the IS. Older subjects (patients + controls) had higher CRP-0h levels ($p=0.021$). There was no significant difference between men and women in terms of their BMI values (26.5 and 24.3, respectively; $p=0.100$).

CRP levels increased significantly during the 24 hours after the IS in four out of five patients with FBTCS particularly after three hours (ANOVA; $p=0.030$; Figures 11 and 12). The overall pattern of CRP change after the IS was U-shaped with an initial decrease in the first three hours which then increased subsequently mainly after 12 hours (Figure 12). In general, the trend analysis showed a significant quadratic trend when all 31 patients were analyzed together ($p=0.029$; Table 11). The quadratic trend was statistically significant in some subgroups of patients such as TLE syndrome and duration of index seizure ≥1min.

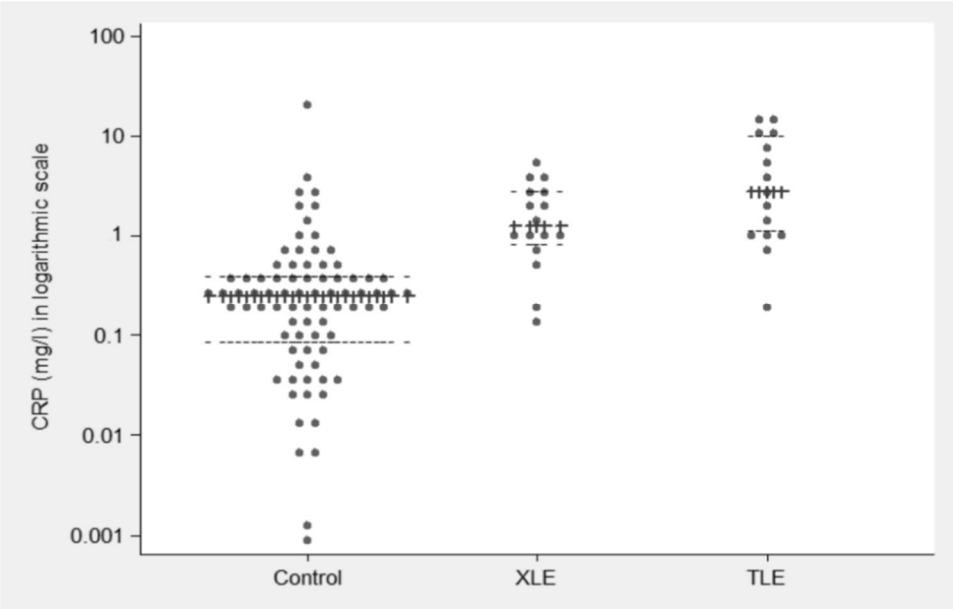

Figure 11. C-reactive protein (CRP) level in healthy controls and patients by syndrome (TLE: Temporal lobe epilepsy, XLE: Extratemporal lobe epilepsy; +++ = median, - - - = interquartile range)

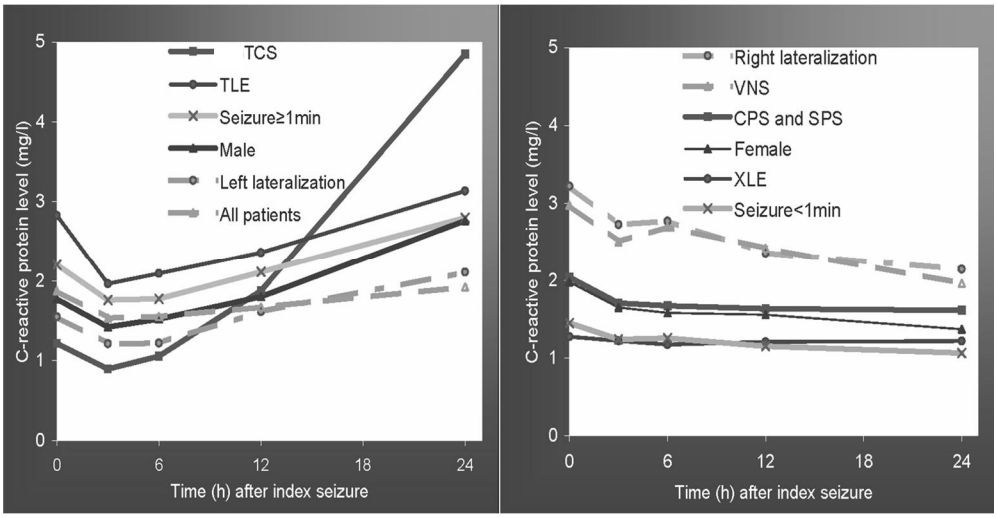

Figure 12. Geometric mean trend of C-reactive protein level at baseline (0h) and 3, 6, 12, and 24 h after index seizure. TCS: focal onset tonic-clonic seizure, TLE: Temporal lobe epilepsy, XLE: Extratemporal lobe epilepsy, VNS: Vagus nerve stimulator

Table 12. C-reactive protein level at baseline, and repeated measurements at 3, 6, 12, and 24 hours after index seizure in the CRP study

		Baseline C-reactive protein (mg/l)					Measurements 0-24 h		H0 to max
	n	Median	Mean	SD	Adj GMR	P LTLR	P ANOVA	P Quadratic	P MWT
All	111	0.3	1.5	3.3		<0.001			
Healthy controls[Ref.]	80	0.3	0.7	2.6	1.0				
Epileptic patients	31	2.0	3.5	4.0	10.1		*0.224[W]*	0.029	
Sex							*0.770[B]*		*0.036*
Female[Ref.]	16	2.0	3.5	4.0	1.0		*0.120[W]*	0.120	
Male	15	1.5	3.5	4.2	1.4		*0.047[W]*	0.024	
Age (mean 34, range 16-58)					1.04	0.033*	*0.003[B]*		*0.451*
≤34 Ref.	17	1.2	2.6	2.9			*0.522[W]*	0.522	
>34	14	2.4	4.7	5.0			*0.369[W]*	0.369	
Epilepsy type						0.378	*0.060[B]*		*0.874*
XLE[Ref.]	16	1.2	2.0	1.7	1.0		*0.888[W]*	0.518	
TLE	15	2.8	5.2	5.1	1.5		*0.222[W]*	0.035	
HS						0.786	*0.920[B]*		*0.953*
TLE without HS[Ref.]	5	2.8	4.9	5.4	1.0		*0.366[W]*	0.196	
TLE with HS	10	2.8	5.3	5.3	0.8		*0.391[W]*	0.125	
Etiology							*0.191[B]*		*0.574*
Unknown[Ref.]	11	2.0	3.5	4.0	1.0		*0.387[W]*	0.122	
HS	10	2.8	5.3	5.3	1.2	0.797	*0.391[W]*	0.125	
Cortical dysplasia	5	1.0	1.2	1.0	0.4	0.112	*0.356[W]*	0.224	
Other	5	2.0	2.4	1.4	1.6	0.448	*0.377[W]*	0.394	
Lateralization							*0.512[B]*		*0.364*
Left[Ref.]	16	1.2	3.4	4.3	1.0		*0.086[W]*	0.032	
Right	9	3.5	4.8	4.6	2.2	0.052	*0.144[W]*	0.850	
Left and right	2	1.4	1.4	0.3	1.3	0.593	*0.649[W]*	0.597	
Unknown	4	2.8	2.2	1.3	1.39	0.674	*0.738[W]*	0.813	
Index seizure type						0.317	*0.884[B]*		*0.005*
Tonic-clonic (FBTCS/GTCS)[Ref.]	5	1.2	2.0	2.1	1.0		*0.030[W]*	0.073	
Focal (FAS, FIAS,FASE)	26	2.0	3.8	4.3	1.7		*0.157[W]*	0.202	
Duration of index seizure (median 1min, range 5s-5min)					0.8	0.267*	*0.160[B]*		*0.081*
<1 min[Ref.]	12	1.6	2.1	1.7			*0.033[W]*	0.739	
≥1 min	19	2.0	4.4	4.8			*0.132[W]*	0.029	
Using enzyme-inducing AED							*0.458[B]*		*0.287*
No[Ref.]	8	1.4	2.4	2.4	1.0		*0.820[W]*	0.937	
Weak (oxcarbazepine)	10	1.9	2.4	2.1	1.1	0.875	*0.179[W]*	0.107	
Strong (CBZ/PHN)	13	2.0	5.1	5.4	1.6	0.385	*0.246[W]*	0.084	

* The variable was used as continuous in the analysis; Adj GMR: Geometric mean ratio [geometric mean in a group divided by mean in the reference (Ref.) group] adjusted for age and sex; LTLR: Log-transformed linear regression adjusted for age and sex; ANOVA: Log-transformed repeated measures analysis of variance (B: Test of between-groups effects, W: Test of within-

groups effects); Quadratic: Quadratic trend analysis; H0 to max: Difference between baseline CRP value and maximum value after seizure; MWT: Mann-Whitney test; AED: Antiepileptic drug; CBZ: Carbamazepine; CPS: complex partial seizure; FAS: focal aware seizure; FASE: Focal aware status epilepticus; FBTCS: focal to bilateral tonic-clonic seizure; FIAS: focal impaired awareness seizure; GTCS: generalized tonic-clonic seizure, HS: Hippocampal sclerosis; PHN: Phenytoin; SD: Standard deviation

Since the IS type displayed a significant interaction in log-transformed repeated measures analyses of variance, the analyses were repeated stratified for each seizure type (test of within-groups effects in Table 11. Mann-Whitney (rank sum) test also confirmed the difference between index seizure type ($p=0.005$) in terms of the maximum difference with baseline CRP level (maximum CRP level among four measurements after the IS minus baseline level). In line with the baseline CRP level, older patients ($p=0.003$) had a significantly higher mean of all five CRP measurements during the baseline and 24 h measurements after the IS (in logarithmic scale; test of between-groups effects in Table 11). In patients with VNS, the CRP level was not higher than in other patients with epilepsy.

5.3 Cell-free DNA concentrations in patients with seizures

The baseline concentrations of cf-DNA were dependent on the type of epilepsy. The mean baseline concentrations of cf-DNA were lower in patients with XLE compared to control subjects (0.72 μg/ml vs. 0.80 μg/ml; $p = 0.001$) (Figure 13, Table 12). There was no significant difference in the concentration of cf-DNA between patients with TLE and control subjects. The concentration of cf-DNA was lower in female patients (0.71 μg/ml) compared to male patients (0.83 μg/ml; $p=0.022$). A similar difference was observed in control subjects; female controls had a lower concentration of cf-DNA (0.76 μg/ml) than males (0.86 μg/ml; $p<0.001$). The baseline concentration of cf-DNA did not differ between patients with BMI fewer than 25 and those with BMI ≥ 25. Other clinical determinants such as patient's age, etiology of epilepsy, duration of epilepsy, or seizure frequency did not have any effect on the baseline concentration of cf-DNA.

The effect of seizure on the concentration of cf-DNA was influenced by the duration of epilepsy and the BMI value (Table 13). When the maximal difference between the baseline and the peak level after IS was analyzed, there was no significant difference in all patients with epilepsy (Figure 14). The maximum

Table 13. Cell-free DNA at baseline.

		Baseline			
	n	Median	Mean	Std. error	P_{LTLR}
All	300	0.79	0.79	0.008	
Healthy controls Ref	250	0.80	0.80	0.008	
Patients with epilepsy	50	0.75	0.77	0.025	0.019 *
Age (mean 32, range 16-58)					
≤32 Ref	25	0.73	0.75	0.034	
>32	25	0.75	0.78	0.036	0.60 *
Epilepsy type					
XLE Ref	23	0.69	0.72	0.027	
TLE	23	0.78	0.82	0.040	0.087
Generalized epilepsy	4	0.78	0.75	0.119	0.46
Lateralization					
Left Ref	21	0.76	0.77	0.044	
Right	14	0.78	0.80	0.035	0.23
Other	15	0.69	0.73	0.043	0.84
Index seizure type					
FBTCS Ref	6	0.71	0.77	0.101	
Focal (FAS,FIAS,FASE)	44	0.75	0.77	0.025	0.61
Duration of epilepsy					
≤18 Ref	24	0.75	0.78	0.035	
>18	25	0.72	0.75	0.036	0.47
Seizures per month last year					
≤10 Ref	26	0.77	0.80	0.040	
>10	24	0.71	0.74	0.027	0.36
Duration of index seizure (sec)					
≤65 Ref	26	0.71	0.75	0.034	
>65	27	0.76	0.79	0.035	0.40

FAS: focal aware seizure; FASE: Focal aware status epilepticus, FIAS: focal impaired awareness seizure; GS: generalized tonic-clonic seizure, FBTCS: focal to bilateral tonic-clonic seizure. LTRL = linear regression models adjusted for age and gender (* adjusted only for gender); outcome = log-transformed cf-DNA

concentration of cf-DNA after baseline measurement was significantly lower in patients whose duration of epilepsy was ≥ 18 years compared to those with a duration of epilepsy < 18 years (0.022 μg/ml vs. 0.031 μg/ml; p = 0.044). The maximum concentration of cf-DNA was higher in patients with a BMI ≥ 25 compared to those with BMI < 25 (0.041 μg/ml vs. 0.004 μg/ml; p = 0.006). The patient's age, epilepsy syndrome, duration of epilepsy, or seizure frequency did not explain the changes of the concentrations of cf-DNA at different time points after IS. The effect of seizure burden on the changes of the concentrations of cf-DNA was not significant.

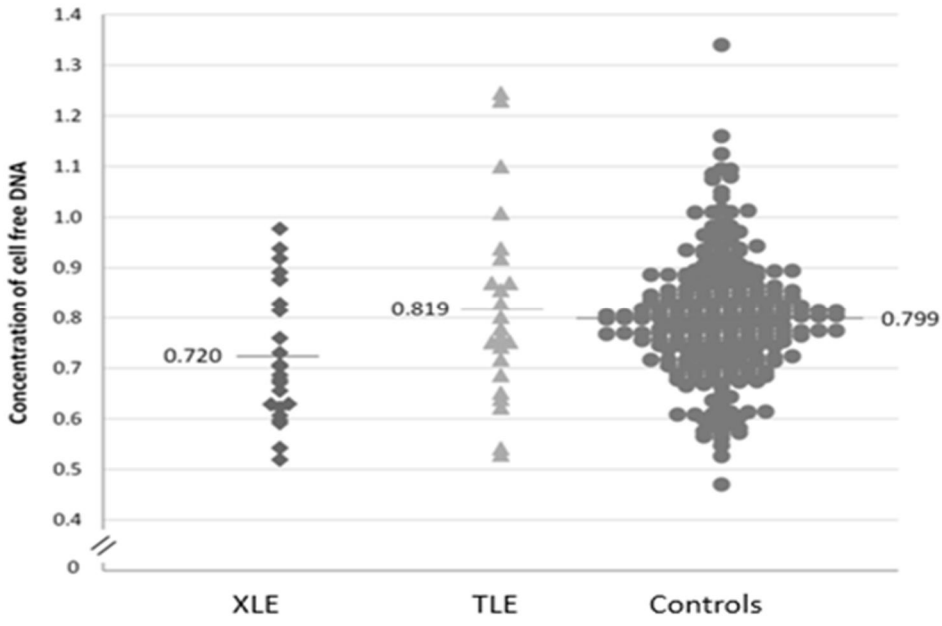

Figure 13. Baseline and mean concentration of cf-DNA in patients with extratemporal lobe epilepsy (XLE), patients with temporal lobe epilepsy (TLE) and control subjects (μg/ml).

Table 14. Difference between maximum and baseline concentration of cell-free DNA

	n	Median	Mean	Std. error	P
Patients with epilepsy	50	0.007	0.025	0.021	
Age					0.79 [1]
≤32	25	0.005	0.036	0.040	
>32	25	0.010	0.014	0.013	
Epilepsy syndrome					0.73 [2]
XLE	23	0.010	0.046	0.038	
TLE	23	0.001	0.004	0.018	
GE	4	0.009	0.026	0.110	
Lateralization of seizure					0.43 [2]
Left	21	0.002	0.009	0.013	
Right	14	-0.004	-0.008	0.022	
Other	15	0.040	0.078	0.064	
IS type					0.49 [1]
FBTCS	6	0.014	0.023	0.048	
Focal (FAS, FIAS, FASE)	44	0.003	0.025	0.023	
Duration of epilepsy					0.044 [1]
≤18	24	0.027	0.031	0.021	
>18	25	-0.005	0.022	0.037	
Seizures per month last year					0.13 [1]
≤10	26	-0.005	0.004	0.022	
>10	24	0.019	0.047	0.036	
Duration of index seizure (sec)					0.077 [1]
≤65	26	0.019	0.061	0.034	
>65	24	-0.005	-0.014	0.021	
Seizure burden[a] (sec)					0.50 [2]
10-99	17	0.005	0.011	0.025	
100-249	17	0.010	-0.013	0.019	
250+	16	0.003	0.080	0.055	
BMI (kg/m^2)					0.006 [1]
<25	22	-0.009	0.004	0.043	
≥25	28	0.021	0.041	0.016	

BMI = body mass index; FAS= focal aware seizure; FASE=Focal aware status epilepticus, FIAS= focal impaired awareness seizure; GE = generalized epilepsy; IS = index seizure; FAS= focal aware seizure, FASE=Focal aware status epilepticus, FIAS=focal impaired awareness seizure; FBTCS: focal to bilateral tonic-clonic seizure; TLE = temporal lobe epilepsy; XLE = extratemporal lobe epilepsy, [1] Mann-Whitney test; [2] Kruskal-Wallis test. [a]During sampling period (Including IS)

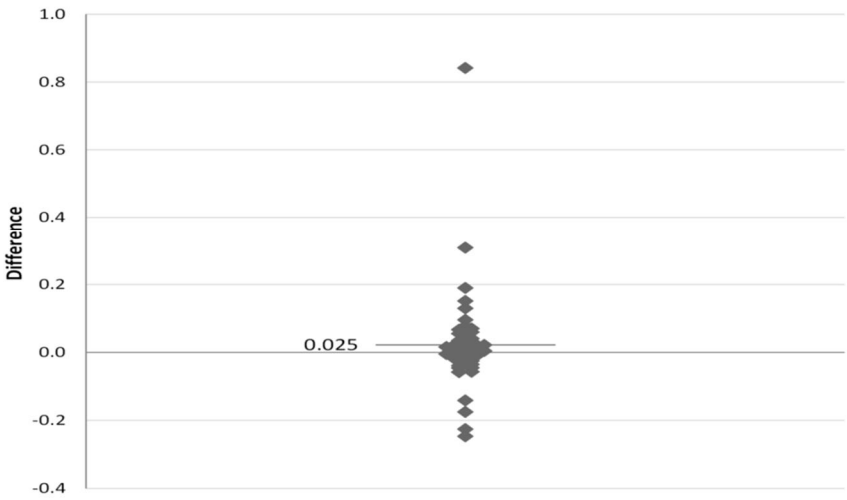

Figure 14. Maximal and mean difference between the baseline and peak concentration of cf-DNA after index seizure (IS) (µg/ml) in study patients.

6 DISCUSSION

The present dissertation provides evidence of seizure-related inflammatory changes in human epilepsy in a well-controlled environment in VEEG monitoring which provides exact information about the extent, localization and duration of seizures. In VEEG, also subclinical seizures that might influence how the inflammatory marker responses are registered (Wang et al. 2017). With this monitoring, it was also possible to exclude acute causes of seizures, e.g. trauma, infections, stroke, which may themselves also induce the production of inflammatory mediators. Drug-resistant epilepsy exerts an enormous influence on an individual's life, and more work will be needed to clarify the related mechanisms. The present dissertation provides evidence from human patients that inflammatory modulators are produced related to seizures in patients with refractory epilepsy evaluated undergoing VEEG monitoring, and furthermore, that the epilepsy type can influence the inflammatory mediators IL-6 and hsCRP, but does not alter the release of cf-DNA related to a single seizure. We were also able to demonstrate that the response to a single seizure in TLE was dependent on the previous seizure frequency and IL-6 concentration. Unfortunately, it was not possible to collect CSF samples. IL-1Ra is markedly, nearly nine times higher, in healthy individuals in plasma than in CSF, and it has been reported that IL-6 is released after seizures, there being as much as five times higher concentrations in CSF than in plasma (Peltola et al. 2000). Despite these limitations, this dissertation attempts to clarify the immunological differences in epilepsy types in well-controlled circumstances.

6.1 The association of baseline characteristics with the seizure response

6.1.1 The assessment of baseline levels of inflammatory markers in epilepsy patients

In patients with focally restricted seizure as the IS, the IL6 baseline concentration was significantly higher compared to patients in whom the IS was a tonic-clonic seizure. The frequency of TCS is much less than FIAS and FAS, thus explaining this difference.

The baseline serum levels of CRP were significantly higher in patients with refractory epilepsy compared to controls. In epilepsy patients increased levels of CRP also correlate with poor visuospatial ability and immediate memory (Hermann et al. 2017) pointing to an association between seizures and the inflammatory load. Epilepsy type may have some influence on CRP, because all five patients with elevated CRP at baseline had TLE, but none of the XLE had elevated values. IL-6 induces CRP and in patients with chronic TLE have higher serum IL-6 levels than patients with XLE (Liimatainen et al. 2009b).

Based on previous large studies from the general population, it is a well-known fact is that the plasma concentrations of CRP and IL-6 are higher in older people (Donato et al., 2008). In our CRP study, older patients and controls had higher CRP baseline levels ($p=0.021$). In line with the baseline CRP level, older patients had a significantly higher mean of all five CRP measurements during the baseline and 24 h measurements after the IS. Higher baseline CRP levels were also detected in those of an older age at diagnosis. Patients with TLE were older than patients with XLE, but there was no difference in baseline CRP levels between the groups.

In patients with epilepsy with strong enzyme-inductors (carbamazepine or phenytoin) the mean CRP baseline levels were twice as high as in patients with non-inducing drugs, but it did not reach statistical significance. Higher CRP baseline levels were associated with lower number of AED. There is not much evidence supporting the assumption that AEDs influence IL-6, CRP or cf-DNA levels. AEDs and their combination may have still an unknown impact on cytokines. Valproic acid or topiramate had no impact on IL-6 or IL-1β serum levels (Sonmez et al. 2013).

Higher CRP baseline levels were also detected in those with an older age at diagnosis. Older patients had higher CRP during baseline and after IS serial measurements. The CRP concentration is known to increase during aging. The baseline CRP level did not show any significant association with sex, etiology, seizures frequency during the year before the VEEG nor with the index seizure type or the duration of the index seizure.

6.1.2 The effect of epilepsy type on baseline cf-DNA levels

Cf-DNA is considered as a marker of inflammation, cell death and degeneration, and increased levels have been found in refractory epilepsy patients as compared to healthy individuals (Liimatainen et al. 2013). The levels of cf-DNA have been found to be elevated in acute neurological conditions such as stroke and TBI and they have also correlated with the severity of the disease and prognosis (Mittra, Nair, Mishra 2012b). Neither the origin nor the exact mechanism is fully understood to explain how cell derived nucleic acids gain access to the circulation but there is accumulating data to suggest that they are released during cell death or they represent an active metabolic released by the cells. In this thesis in patients with refractory patients, the epilepsy type exerted an influence on cf-DNA interictally; levels of baseline cf-DNA were lower in patients with XLE, but not in TLE, in comparison to the levels in nonepileptic controls. A previous study in subjects with refractory focal epilepsy did not report a similar finding; the majority of patients had elevated interictal levels of cf-DNA in TLE with or without HS and XLE (Liimatainen et al. 2013). There is no detailed explanation for our findings of lower levels after IS in XLE. The TLE patients were older than the XLE patients (40 vs 30 years), and there might be excessive consumption of cf-DNA either based on epilepsy type or younger age. In older patients with TLE, there may be already apoptosis and atrophy; for that reason, a single seizure does not release cf-DNA into the circulation. In cases of sepsis, higher plasma cf-DNA levels have been associated with a poor outcome (Huttunen et al. 2011). Inflammation is also important for tissue repair, and cf-DNA can activate the innate immune system and a strong correlation exists between the level of circulating nucleosomes, IL-6 and TNF-α (Mittra, Nair, and Mishra 2012a).

The baseline concentration of cf-DNA was lower in female patients (0.71 μg/ml) compared to male patients (0.83 μg/ml; p=0.022). A similar difference was

observed in control subjects; female controls had a lower concentration of cf-DNA (0.76 μg/ml) than males (0.86 μg/ml; $p<0.001$). In this study, the epilepsy syndrome, patient's age, duration of epilepsy, or seizure frequency during the last year had no influence on the changes of the concentration of cf-DNA at different time points after IS.

6.2 Seizure-induced production of inflammatory markers

6.2.1 The association of seizure type with inflammatory response

In earlier studies investigating IL-6, it has been claimed that a low concentration of this cytokine can be found in a healthy brain (Alyu and Dikmen 2016; Lehtimäki et al. 2003), and after tonic-clonic seizures, its levels are known to increase in CSF and blood serum (Lehtimäki et al. 2004; Lehtimäki et al. 2010; Peltola et al. 1998; Peltola et al. 2000). There is also a clear correlation between the IL-6 concentration in CSF and plasma. When compared to controls, there are higher IL-6 concentrations in CSF in patients with acute symptomatic seizure in comparison with plasma concentrations; the plasma levels of IL-6 are most likely originated in the central nervous system via venous drainage (Peltola et al. 1998; Peltola et al. 2000).

IL-6 is a cytokine which is known to be rapidly upregulated as a response to tissue trauma and inflammation. Experimental results originating from a variety of animal models have established that there is an acute temporal release of IL-6, with initial detection at 1 h, followed by a peak concentration between 2 and 8 h after the injury (Morganti-Kossmann et al. 2007). The significance of the IL-6 activation in epilepsy has been less extensively studied than the IL-1 family of cytokines. IL-6 is considered to be a proconvulsant cytokine (Kalueff et al. 2004), but also neuroprotective properties have been reported (Penkowa et al. 2001). There is experimental evidence from rodent models for a rapid inflammatory response in glia after a seizure (De Simoni et al. 2000; Vezzani et al. 2015b).

A previously published study with a smaller patient group has revealed that plasma IL-6 levels were increased more often after secondary generalized seizures than after focal seizures with impaired awareness in VEEG (Lehtimäki et al., 2007);

and in emergency room studies, IL-6 levels were strongly elevated after recurrent tonic-clonic seizures in serum and CSF, whereas after single tonic-clonic or prolonged focal seizures (aware or impaired awareness), the levels were increased to a lesser extent (Lehtimäki et al. 2004; Lehtimäki et al. 2010). The results emerging from this dissertation are in line with those reports; the present study with a larger patient population detected increased levels of IL-6 at all time points between 3h and 24h after the IS compared to the baseline. The increase in the IL-6 levels was more prominent after the more severe seizure types, tonic-clonic seizures, at the 3 h and 6 h time points compared to only focally limited minor seizures, which is in line with experimental TBI results. Uludag et al 2013 reported no difference in the postictal IL-6 levels between seizure types, but in that study, most of the patients, i.e. 17 patients, had TCS, whereas only six patients had focally limited impaired awareness seizures (Uludag et al. 2013).

The CRP study found that after a single seizure, the most important predictor of an increase in the CRP level was that the seizure had been of the tonic-clonic seizure type. IL-6 may be one of inducers of CRP. The present dissertation detected increased levels of IL-6 at all time points between 3h and 24h after the IS as compared to the baseline. There were not such prominent findings in CRP levels, but more severe seizures, i.e. FBTCS, increased the levels significantly after the IS, particularly after 3 h. The increase of IL-6 levels was also more prominent after the more severe seizure types at the 3h and 6h time points.

In TCS, the seizure activity spreads throughout the brain, whereas in FAS and FIAS, the epileptic activity is restricted to the seizure focus and associated structures, and there may be less leakage through the BBB into plasma. Unlike previous reports (Lehtimäki et al. 2007), in the present study, the postictally elevated IL-6 levels had still not returned to the baseline level within 24 hours. Postictal IL-6 levels remained elevated also in another VEEG study (Bauer et al. 2009; Lehtimäki et al. 2011). Uludag et al examined patients with a predominantly TCS type; they reported higher plasma IL-6 and IL-1Ra levels at 12 h than at baseline (Uludag et al. 2013). Bauer et al 2009 observed non-significantly slightly higher IL-6 levels after TCS than in focal-restricted seizures, and also detected higher levels after right-sided seizures as compared to their left-sided counterparts (Bauer et al. 2009); in contrast in our study, there was no difference between the lateralization of the seizure focus and furthermore we did not find any difference which could be attributable to lateralization.

In the present thesis the increase of IL-6 and CRP levels was more prominent after the more severe seizure types suggesting higher inflammatory response in these seizure types.

6.2.2 The association of epilepsy type with the inflammatory responses

The effects of single reliably analyzed seizures on IL-6 in patients with chronic refractory epilepsy have been investigated earlier in a few studies with a relatively small number of study patients; IL-6 levels increased after a seizure without there being any difference between TLE and XLE (Bauer et al. 2009; Ishikawa et al. 2015; Lehtimäki et al. 2007; Uludag et al. 2013). In our final cytokine study with 49 patients, the major finding was an increased production of IL-6; its levels were significantly increased at all time points between 3 h and 24 h after the IS compared to the baseline when all seizures were analyzed, in confirmation of earlier results. In the previous study with 11 patients with chronic localization-related epilepsy, the levels IL-6 and IL-1Ra were increased after seizures, whereas those of soluble IL-6 receptors (sIL-6R and Gp130) and IL-1β remained unchanged in plasma after single seizures during VEEG recordings (Lehtimäki et al. 2007). That small study demonstrated that single seizures can evoke a significant change in IL-6 and IL-1Ra plasma levels, but because of the limited sample size, the importance of the epilepsy or seizure type could not be addressed.

The present thesis provides additional data to highlight the differences between TLE and XLE in seizure-related responses on cytokine inflammatory markers. In our pilot cytokine study and in the other previous study with smaller patient groups, increased plasma IL-6 levels were measured at the 6 h time point after the IS (Lehtimäki et al. 2007). We found evidence to support the concept that the epilepsy type would determine the response; an increase in plasma levels of proinflammatory IL-6 was observed only in the TLE group. Bauer et al found that the levels of IL-6 increased 1 h postictally and remained elevated for 24 h, all of the 25 patients in that trial had TLE with FIAS or FBTCS (Bauer et al. 2009). In two other VEEG studies (one with 23 and the other with 12 refractory epilepsy patients), levels of IL-6 were increased in both patients with TLE and XLE. Furthermore, in a substantially larger number of patients in our final cytokine study, the maximum elevation in the serum IL-6 levels after IS was significantly greater in TLE than in XLE (Ishikawa et al. 2015; Uludag et al. 2013). The findings

in our cytokine studies i.e. elevated levels IL-6 and the trend of IL-1Ra to decrease after the seizure in refractory TLE support the previous finding after FSE that a lower ratio of IL-1Ra/IL-6 is associated with acute hippocampal injury (Gallentine et al. 2017). There have been no large-scale studies conducted under well-documented circumstances investigating these seizure-related changes in patients with refractory epilepsy.

In our subsequent pilot VEEG study with 20 patients, postictal levels of IL-1Ra and IL-1β did not significantly differ from baseline levels in either of the patient groups; but there was a decreasing trend from 12 to 24 hours. In the experimental study in a mouse model of pediatric brain injury, IL-1Ra treatment mice showed reduced seizure susceptibility and a reduction in HS during the subacute period, and after a latency period IL-1Ra treated mice suffered fewer seizures compared to controls, and they had also greater preservation of cortical tissue (Semple et al. 2017). In the previous studies, patients with chronic refractory epilepsy have shown a proinflammatory cytokine profile in plasma (high IL-6, low IL-1Ra and low IL-1Ra/IL-1β ratio) (Hulkkonen et al. 2004; Liimatainen et al. 2009a). Uludag et al 2013 reported that the IL-1β/IL-1Ra ratio decreased 3 h postictally and IL-1Ra increased at 12 h, but there was no correlation between epilepsy type with the levels of IL-6, IL-1Ra, IL-β or with the IL-1β/IL-1Ra seizure-related response (Uludag et al. 2013). In the other VEEG studies with chronic epilepsy, the levels of IL-1Ra were increased after seizures whereas those of IL-1β remained unchanged in plasma after single seizures during VEEG recordings (Ishikawa et al. 2015; Lehtimäki et al. 2007; Uludag et al. 2013). In the thesis, the finding that IL-1Ra decreased from 12-24 h in TLE but not XLE, differs from earlier reports.

The production of IL-1Ra is mainly activated by IL-1β, so it is possible that upregulation of IL-1Ra reflects the activation of the IL-1 system. On the other hand, IL-1β is one of the main inducers of IL-6 (Dinarello 1996), but there are experimental findings that it is released only locally in the brain (Lehtimäki et al. 2003; Ravizza et al. 2008b). IL-1β is proconvulsant and causes neuronal damage, whereas IL-1Ra counteracts the IL-1 system and it has been suggested that IL-1Ra could act as an anticonvulsive and neuroprotective agent (Vezzani et al. 2000). IL-1β is upregulated after experimental seizures, but similar to the results of this thesis, previous clinical studies have not been detected any differences in IL-1β concentrations after the seizures (Peltola et al. 1998; Peltola et al. 2000). This is

most likely explained by the findings that the increased expression of IL-1β takes place mainly in the hippocampal area which does not have any appreciable effect on the levels of soluble cytokines either in the CSF or blood circulation.

The epilepsy type influenced the CRP responses. A quadratic trend was found in CRP after IS in TLE, whereas there were no changes in XLE. FBTCS evoked increased IL-6 levels, which is known to be one of the major inducers of CRP, so these results are consistent. In one report, interictal CRP was higher in IGE compared to healthy controls, but there was no difference between patients with AED and untreated patients (Liguori et al. 2017); in that study, GTCS was also the dominant seizure type.

Mesial TLE is the most commonly studied focal epilepsy type, and the temporal lobe is vulnerable in many conditions affecting the brain. There is convincing evidence to suggest that TLE may be a progressive disease and lead to hippocampal atrophy (Pitkänen and Sutula 2002). Peripheral IL-6 activation may be involved in this insidious pathology. In a cross-sectional study, the serum levels of IL-6 were chronically elevated in epilepsy patients compared to healthy controls, and serum levels of IL-6 were higher in patients with TLE compared to those with XLE (Liimatainen et al. 2009b). In this thesis, new data is that the IL-6 response to a single seizure is different in TLE in comparison with XLE; there was no difference between TLE with or without HS. In human tissue, HS was not associated with the elevation in the levels of increased pro-inflammatory cytokines including IL-6 (Aalbers et al. 2014), thus there may be mechanisms other than HS explaining the peripheral inflammatory profile in TLE. If in TLE, the IL-6 concentration is constantly elevated, the levels of this cytokine cannot be further augmented by a single seizure. If the IL-6 level is low, the seizure induces IL-6 production in TLE but not in XLE, and it may be attributable to the ongoing active inflammation process in TLE.

DNA release following cell death and active secretion of DNA from cells are major sources of cf-DNA (Mittra, Nair, and Mishra 2012b). Nevertheless, in our study, the cf-DNA levels in TLE did not differ from XLE after the seizure.

6.2.3 The effect of seizure duration on inflammatory response

A longer duration of seizure (≥1 min) increased CRP concentrations postictally. Patients experiencing longer seizures lasting at least 100 seconds had also elevated

IL-6 levels postictally compared with the patients whose seizures were shorter than 100 seconds. CRP is produced in response to inflammatory signals, most prominently to IL-6 (Mantovani et al. 2008), thus the findings in this thesis support the published literature. The higher the total seizure burden during the recording, the greater was the IL-6 response. This supports the putative pro-inflammatory feature of IL-6. The seizure burden exerted no significant effect on the changes in the concentrations of cf-DNA.

6.2.4 The association of pre-seizure seizure frequency with inflammatory response

In this thesis, the major new finding was that the response to a single seizure in TLE was dependent on the previous seizure frequency and on IL-6 interictal concentration. There was no elevation in IL-6 levels after a single seizure in patients with frequent seizures; instead these patients displayed chronically elevated IL-6 concentrations. In contrast, patients with infrequent seizures and who displayed low baseline IL-6 levels exhibited a significant elevation in this cytokine's level after a single seizure.

6.2.5 The association of baseline IL-6 concentration with seizure-related IL-6 response

In an earlier cross-sectional study, there was a subgroup of patients with TLE, who had IL-6 levels over 5 pg/ml, but all of the patients with XLE or healthy controls had levels below 5 pg/ml (Liimatainen et al. 2009b). In this dissertation, in patients with a low baseline IL-6 level (under 5 pg/ml), seizures induced a higher maximum elevation in both absolute and relative values of IL-6 in TLE patients but not in those with XLE. This was not the case if the TLE or XLE patient had a high baseline IL-6 concentration. As far as we are aware, this is the first study to demonstrate that lower chronic IL-6 levels in TLE are associated with a more profound IL-6 response after a single seizure. In contrast, if the baseline IL-6 level was already high, there was no dramatic IL-6 response after the seizure. One possible explanation for these results may be that the chronic high inflammatory load does not undergo a prominent response after a single seizure, and conversely,

a low chronic level induces immunological reactions postictally, and this may mean that TLE has a different immunological background in TLE from XLE.

6.2.6 Other factors influencing on seizure-induced inflammatory markers

Refractory epilepsy patients with a longer duration of disease had lower postictal cf-DNA levels. There may be changes in the neuronal network that influence the response to a single seizure in the long term. The patients with a shorter duration of epilepsy (≤ 18 years) were younger than the patients with a longer duration (>18 years) (28±9 vs. 41±10 years); in addition to the age-related changes there might be modulation of the activation of acute phase reaction, and furthermore, it is possible that a seizure might induce rapid consumption and down-regulation of harmful mediators. While levels of IL-6 appear to be clearly increased both in serum and CSF after GTCS in humans, levels of soluble receptor IL-6R decreased (Lehtimäki et al. 2004).

The effect of the seizure on the concentration of cf-DNA was influenced also by BMI. The maximum concentration of cf-DNA was higher in patients with body mass index (BMI) ≥ 25 compared to those with BMI < 25. This may reflect a higher risk of cardiovascular disease.

6.3 The clinical significance of seizure-dependent immunoactivation in refractory epilepsy

In experimental studies, SE has increased the expression of inflammatory cytokines in the brain. These experimental studies have indicated IL-6 to be a proconvulsant compound (Kalueff et al. 2004). In addition, IL-6 has been postulated to exert neuromodulatory properties which lead to alterations in neuronal network excitability (Vezzani and Viviani 2015). A chronic low-grade proinflammatory state may reflect the severity of the disease in epilepsy patients. Increased CRP and IL-6 levels have been associated with increased mortality in elderly patients (Jylhävä et al. 2012; Hermann et al. 2017). A higher GTCS frequency increases greatly the risk for SUDEP (Hesdorffer et al. 2012). In a small neuropathology study of SUDEP, there were no signs of greater inflammatory reactions or BBB leakage in SUDEP

subjects as compared to controls (Michalak et al. 2017); thus the mechanism of SUDEP is still unclear.

IL-6 may be one of the general markers of local and systemic immune system activation in specific subtypes of patients with epilepsy, and therefore it is important to clarify in more detail the diverse factors that influence IL-6 responses. There was no difference in interictal or ictal CRP or IL-6 levels between patients with HS and other etiologies. This finding is supported by an earlier human study from Aalbers et al. with surgically resected hippocampi from patients with medically refractory TLE (Aalbers et al. 2014), i.e. there was no difference in the levels of IL-6, IL-1α, IL-1β, IL-1Ra or IL-10 in hippocampal expression in HS and non-HS. Thus, it seems that HS does not account for the difference in immune responses between TLE and other epilepsies. However, this dissertation would have been more relevant if a wider range of cytokines could have been explored.

AEDs are used to prevent seizures, but they do not prevent the underlying epileptic pathology (Moshé et al. 2015), and therefore a better understanding of complex and multifaceted factors underlying epileptic disorders will be necessary for developing strategies for more successful treatment of medically refractory epilepsy. By switching enzyme-inducing AEDs to levetiracetam, lamotrigine, topiramate or zonisamide, it was possible to reduce the CRP-levels of epileptic patients in serum (Mintzer et al. 2009, Mintzer et al. 2012, Mintzer et al. 2016). After levetiracetam treatment, serum levels of hsCRP and S100B protein were lower (Chen et al. 2015), patients treated with levetiracetam displayed decreased $CD8^+$ cell counts (Nowak et al. 2010), i.g. one could speculate that levetiracetam may have anti-inflammatory properties. In patients taking valproate, increased numbers $CD4^+$ T lymphocytes have been observed (Nowak et al 2010), but also lower levels of CRP have been reported (Yuen et a. 2010). In our study, there was no statistically significant difference between patients taking enzyme inducing drugs and controls, perhaps due to the small sample size. The influences of other AEDs were not studied.

The present data support a role for inflammation in human epilepsy. In this thesis, the major new finding was that the response to a single seizure in TLE was dependent on the previous seizure frequency and also on the interictal IL-6 concentration. We confirmed a previous finding that seizures elevated the IL-6 concentration postictally. It was observed that the maximum change in the IL-6 level after the IS was significantly greater in TLE than in XLE. We also noted that

the seizure type exerted an effect on baseline IL-6 levels, i.e. IL-6 levels were higher in patients with tonic-clonic seizures. IL-1β is one of the main inducers of IL-6 (Dinarello 1996), and IL-1β has been associated with seizure susceptibility in FS both in rats (Dubé et al. 2010) and in humans (Virta et al. 2002, Yu et al. 2012).

The potential role of cf-DNA in neurological diseases and especially in epilepsy is not well established. After a seizure cell damage may be reversible, consequently cf-DNA is not released. This may be similar to the situation in myocardial infarction which begins with reversible ischemia; it is only prolonged ischemia that leads to infarction and necrosis (Chang et al. 2003). It would be interesting to study cf-DNA levels after status epilepticus. Earlier studies have demonstrated that a high lifetime seizure number and a longer duration of focal epilepsy contribute to more severe cumulative neuronal damage (Briellmann et al. 2002). We did not find any correlation between the duration of epilepsy and the increase in the concentrations of IL-6 and CRP. A longer duration of epilepsy was associated with lower levels of cf-DNA after IS pointing to an irreversible change in the inflammatory profile.

6.4 Possibilities for therapeutic application in inflammatory-related epilepsy

Immunomodulatory therapies (corticosteroids, plasma exchange, intravenous immunoglobulin) have been used in the treatment of refractory and super-refractory status epilepticus, especially when autoimmune encephalitis has been diagnosed or suspected (Bauer et al. 2017; Zeiler et al. 2016; Zeiler et al. 2017). There are experimental studies which have targetted specific parts of the inflammatory system (Vezzani et al. 2015b). An injection of IL-1Ra, a drug used in autoimmune disorders, reduced both the incidence and the severity of SE in rats, and the onset of SE was delayed (Marchi et al. 2009). A pharmacological blockade of the IL-1β by human recombinant IL-1RA (anakinra) was able to reduce cell loss in the forebrain areas after SE (Noé et al. 2013). In pediatric patients glucocorticosteroids decreased the seizure frequency by at least 50 % or interrupted SE (Marchi et al. 2011). MicroRNAs are gene regulators which may have a potential role in the regulation of the inflammatory pathways in epilepsy,

e.g. they may target IL-1Ra, IL-6, IL-1β, or the adhesion molecule ICAM-1 (Srivastava et al. 2016). Recurrent seizures have also been shown to lead to chronic expression of VCAM-1 with a subsequent change in BBB permeability (Fabene et al. 2008). In one case report of a patient with multiple sclerosis and focal epilepsy, inhibition of leukocyte adhesion by administration of natalizumab, reduced both seizure frequency and seizure severity (Sotgiu et al. 2010).

Effective therapies for preventing epileptogenesis or treating drug-resistance epilepsy are still lacking, which emphasizes the need to acquire more knowledge also from human studies. The development of specific biomarkers for different epilepsies, seizure types and syndromes as well markers for the progression of epileptogenesis could help in clinical diagnosis and practice.

6.5 Limitations of the study

Our study and conclusions are confounded by several factors. Our results are based on a rather large population and the study design required about four years for collecting samples, but in statistical terms this is still a small patient group. It is, however, important to identify and examine a well-defined group of epilepsy patients with such an information-intensive technique as VEEG.

The classification of patients and the localization of the seizure focus were based on careful clinical, scalp EEG and radiological assessment, but depth recordings were not available. A different distribution of left- and right-sided foci as well as varying etiologies of epilepsy in our two patient populations could contribute to the variance in the findings between the TLE and XLE. One limitation of our study is that we recruited only a few patients in the idiopathic generalized epilepsy group, making it more difficult to draw definitive conclusions concerning that epilepsy type. There was a misclassification of focal and GTCS seizure types in cf-DNA study but this had no impact on the results. In future studies, larger groups of patients will be needed within the different epileptic syndromes and types to assess whether the induction of IL-6 and IL-1Ra is associated with distinctive etiological forms of epilepsy. Moreover, it remains to be elucidated whether genetic factors such as genetic polymorphisms have any effect on the varying kinetics of circulating IL-6, IL-1Ra and IL-1β in TLE and XLE. There also may be different cytokine gene polymorphisms influencing different

inflammatory responses, but genes were not studied in this thesis. Genetic polymorphisms have been reported to be associated with a higher basal CRP level (Delongui et al. 2017; Soydas et al. 2016), and an analysis of specific gene polymorphisms in our epilepsy patients would have been enlightening and possibly revealed differences between controls and epilepsy patients, but unfortunately it was not able to extend this research project to incorporate genetic testing.

Increased CRP has been a risk factor for in-hospital mortality in patients with cardiovascular disease (Yoshinaga et al. 2017) and in acutely hospitalized multimorbid elderly patients (Nouvenne et al. 2016). In this thesis, the patients were not followed-up to assess the prognosis.

Anticonvulsant medication was reduced in an individual stepwise fashion during the EEG-monitoring. It was not possible to evaluate the effects of the individually reduced AEDs on the levels of the cytokines, more studies are needed to answer this question.

Circadian rhythm of IL-6 has also been noticed, with plasma peaks at 4 a.m. and 4 p.m. (Agorastos et al. 2014), and this may influence serum IL-6 levels, but on the other hand, we collected samples for 24 h, and naturally patients had their seizure different times, both during day and night.

Analysis of CSF would have added some more data to the present studies, but unfortunately, it was not possible to collect CSF samples during VEEG monitoring.

6.6 Future implications

Futures studies both in animals and in humans should be conducted in order to acquire a better understanding about both the transient and the chronic neuroinflammation associated with seizures and different epilepsies types with various etiologies. The present study was unable to address the effect of distinct AEDs due to the large number of patients on polytherapy, making any analysis unfeasible. Futhermore, we did not perform genetic studies, which would have been able to add a new dimension to the interpretation of the immunological responses. In addition to the inflammatory parameters studied in the present thesis, a large and increasing number of potentially interesting other immunological markers, such as IL-10 and IL-17 should be examined in the future. Another

interesting approach would be to conduct a network analysis on the relationships and regulatory factors impacting on the measured inflammatory parameters. In the future, the inflammatory mediators in epilepsy should be investigated from a wider perspective to reveal new therapeutic targets in the devastating disease.

7 SUMMARY

Epilepsy is a chronic disease with differing phenotypes with physical, psychological, emotional and social consequences for affected individuals and their families. The mechanisms involved in the pathogenesis of epilepsy are not fully recognized. Early prevention of epileptogenic conditions is a research goal for the future, but this will require a more accurate understanding of the pathological processes underlying these epilepsies. This thesis with VEEG is valuable, because in humans, the short- and long-term influences on the brain are more difficult to quantify than in animal studies, where tissue analysis are more easily available. Inflammatory changes may have a crucial role. It is likely challenging to clarify the circumstances when the proinflammatory cytokine load is high and prolonged. It is unclear what role inflammatory mediators play in the mechanisms underpinning pharmacoresistance.

The present study provides evidence in human patients about seizure-related changes in serum IL-6, IL-1β, IL-1Ra, CRP and cf-DNA levels. The study was implemented in a well-controlled environment and seizure type was reliably evaluated. The data emerging from this thesis is that patients with frequent seizures displayed a smaller increase of IL-6 after single seizures compared with those with infrequent seizures. Thus, the response to a single seizure may be dependent on the previous seizure frequency and IL-6 interictal levels in patients with refractory epilepsy in TLE but this is not the case in XLE, in which there are usually more frequent seizures. The findings of the present series of studies support the hypotheses that there are immunological seizure-related changes in refractory epilepsy, and furthermore, in TLE and XLE, the immunological pattern is different, which may have an influence on the treatment of epilepsy. TLE differs from XLE in several ways: the mesial temporal lobe is a most vulnerable structure considering the risk of epileptogenesis, and in addition, limbic and autoimmune encephalitis injuries especially affect the temporal lobe, and finally in superrefractory status epilepticus, there are pathological changes more often in the

temporal lobe area observed in MRI. There were no differences in the measured inflammatory markers between TLE with or without HS in this thesis. It is also possible for a TLE patient to have HS although there are no signs of HS in MRI, and in future with advancing techniques, it is likely that the the accuracy of the diagnosis of HS will improve. Nowadays AEDs are used based on epilepsy syndrome, etiology of epilepsy and seizure types, but perhaps in the future, the optimal treatment decision will also need to take into account whether the epilepsy is TLE or XLE. One can also speculate that in the future also inflammatory process will receive more attention in treatment strategies for intractable epilepsy.

One major challenge will be to define inflammatory biomarkers which could help the clinician to prescribe a specific immune-modulatory treatment. In refractory epilepsy, there seem to be differences in temporal and extra-temporal epilepsies also in the peripheral inflammatory responses, but further studies will be needed before clinicians can rely on inflammatory markers for diagnostic, prognostic, and therapeutic purposes. A better understanding of the immunological mechanisms of pharmacoresistant epilepsy may help to target the anti-inflammatory treatment in epileptogenesis. In patients treated with immunotherapeutic interventions, those displaying GAD-TLE experienced a worse outcome and more poor seizure response than patients with antibodies to voltage gated potassium channel (VGKC) (Malter et al. 2015), so there may be different immunological mechanisms and responses in different epilepsies. In an earlier cross-sectional study with chronic epilepsy patients, elevated levels of IL-6 were more prevalent in patients with TLE compared to patients with XLE (Liimatainen et al. 2009b). A recent meta-analysis also demonstrated elevated serum IL-6 levels in TLE patients (de Vries et al. 2016), suggesting that the epilepsy type is important in determining whether or not there is overproduction of cytokines in refractory focal epilepsy. In addition, IL-6 has been postulated to possess neuromodulatory properties which lead to alterations in neuronal network excitability (Vezzani and Viviani 2015). In this thesis, it was observed that the maximum change in the IL-6 level after IS was significantly greater in TLE than in XLE. TLE seems to be a key determinant in the IL-6 response. IL-6 may be one of the general markers of local and systemic immune system activation in specific subtypes of patients with epilepsy, and therefore it is important to clarify in more detail the diverse factors that influence IL-6 responses. However, this dissertation would have been more relevant if a wider range of cytokines had been explored.

In the future, a combination of several biomarkers together with advanced imaging techniques and genetic testing might help to predict epileptogenesis and progression of epilepsy although sufficient sensitivity and specificity will be needed. EEG, combined with functional MRI, could also identify seizure-related networks and help to identify target-specific treatments. The current AEDs are evidently not satisfactory in the treatment of refractory epilepsy; more extensive efforts should be expended to target the treatments more accurately so that therapy can be tailored to the underlying pathological epileptic network.

Considering the complexity of the pathogenesis of epilepsy, it is unlikely that a single biomarker could alone predict the outcome. In the future, it may be interesting to examine whether the responses of the cytokines differ in antibody-related refractory epilepsies and clarify the backgrounds to various responses and thus to find the best optimal immunotherapies for different epilepsies.

8 CONCLUSIONS

- In this thesis, a major new finding was that the response to a single seizure in TLE was dependent on the previous seizure frequency and on the IL-6 interictal concentration.
- The present study, with a large patient population, detected increased levels of IL-6 at all time points between 3 h and 24 h after the IS compared to the baseline in patients with refractory epilepsy.
- The increase of IL-6 and CRP levels after the seizure was more prominent after the more severe seizure types.
- Furthermore, this detailed characterization of IL-6 responses may open new avenues for immunomodulatory interventions in the future.
- The CRP study, with a larger patient population, detected increased levels of IL-6 at all time points between 3 h and 24 h after the seizure in comparison to the baseline.
- The baseline serum levels of CRP were higher in patients with refractory epilepsy as compared to controls.
- Taken together, both the IL-6 and CRP data strengthen the concept that chronic seizures induce peripheral and presumably also CNS inflammation highlighting the need for further studies to clarify this evolving field.

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10 REFERENCES

Proposal for revised clinical and electroencephalographic classification of epileptic seizures. from the commission on classification and terminology of the international league against epilepsy. 1981. Epilepsia 22(4):489-501.

Aalbers MW, Rijkers K, Majoie HJ, Dings JT, Schijns OE, Schipper S, De Baets MH, Kessels A, Vles JS, Hoogland G. 2014. The influence of neuropathology on brain inflammation in human and experimental temporal lobe epilepsy. J Neuroimmunol 271(1-2):36-42.

Abbott NJ, Patabendige AA, Dolman DE, Yusof SR, Begley DJ. 2010. Structure and function of the blood-brain barrier. Neurobiol Dis 37(1):13-25.

Abdijadid S, Mathern GW, Levine MS, Cepeda C. 2015. Basic mechanisms of epileptogenesis in pediatric cortical dysplasia. CNS Neurosci Ther 21(2):92-103.

Agorastos A, Hauger RL, Barkauskas DA, Moeller-Bertram T, Clopton PL, Haji U, Lohr JB, Geracioti TD, Jr, Patel PM, Chrousos GP, et al. 2014. Circadian rhythmicity, variability and correlation of interleukin-6 levels in plasma and cerebrospinal fluid of healthy men. Psychoneuroendocrinology 44:71-82.

Allers K, Essue BM, Hackett ML, Muhunthan J, Anderson CS, Pickles K, Scheibe F, Jan S. 2015. The economic impact of epilepsy: A systematic review. BMC Neurol 15:245,015-0494-y.

Alvim MK, Coan AC, Campos BM, Yasuda CL, Oliveira MC, Morita ME, Cendes F. 2016. Progression of gray matter atrophy in seizure-free patients with temporal lobe epilepsy. Epilepsia 57(4):621-9.

Alyu F and Dikmen M. 2016. Inflammatory aspects of epileptogenesis: Contribution of molecular inflammatory mechanisms. Acta Neuropsychiatr :1-16.

André VM, Cepeda C, Vinters HV, Huynh M, Mathern GW, Levine MS.2008. Pyramidal cell responses to γ-aminobutyric acid differ in type I and type II cortical dysplasia. J Neurosci Res 86:3151-62.

Asadi-Pooya AA, Stewart GR, Abrams DJ, Sharan A. 2017. Prevalence and incidence of drug-resistant mesial temporal lobe epilepsy in the united states. World Neurosurg 99:662-6.

Armitage PA, Farrall AJ, Carpenter TK, Doubal FN, Wardlaw JM. Use of dynamic contrast-enhanced MRI to measure subtle blood-brain barrier abnormalities. Magn Reson Imaging. 2011; 29:305–314.

Ba-Diop A, Marin B, Druet-Cabanac M, Ngoungou EB, Newton CR, Preux PM. 2014. Epidemiology, causes, and treatment of epilepsy in sub-saharan africa. Lancet Neurol 13(10):1029-44.

Bagla R and Skidmore CT. 2011. Frontal lobe seizures. Neurologist 17(3):125-35.

Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Chambless LE, Myerson M, Wu KK, Sharrett AR, Boerwinkle E. 2005. Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident ischemic stroke in middle-aged men and women in the atherosclerosis risk in communities (ARIC) study. Arch Intern Med 165(21):2479-84.

Barkovich AJ, Guerrini R, Kuzniecky RI, Jackson GD, Dobyns WB. 2012. A developmental and genetic classification for malformations of cortical development: Update 2012. Brain 135(Pt 5):1348-69.

Bauer J, Becker AJ, Elyaman W, Peltola J, Ruegg S, Titulaer MJ, Varley JA, Beghi E. 2017. Innate and adaptive immunity in human epilepsies. Epilepsia 58 Suppl 3:57-68.

Bauer S, Cepok S, Todorova-Rudolph A, Nowak M, Koller M, Lorenz R, Oertel WH, Rosenow F, Hemmer B, Hamer HM. 2009. Etiology and site of temporal lobe epilepsy influence postictal cytokine release. Epilepsy Res 86(1):82-8.

Beleza P. 2012. Acute symptomatic seizures: A clinically oriented review. Neurologist 18(3):109-19.

Beleza P and Pinho J. 2011. Frontal lobe epilepsy. J Clin Neurosci 18(5):593-600.

Beleza P. 2009. Refractory epilepsy: A clinically oriented review. Eur Neurol 62(2):65-71.

Benveniste EN. 1992. Inflammatory cytokines within the central nervous system: Sources, function, and mechanism of action. Am J Physiol 263(1 Pt 1):C1-16.

Berg AT and Scheffer IE. 2011. New concepts in classification of the epilepsies: Entering the 21st century. Epilepsia 52(6):1058-62.

Berg AT, Berkovic SF, Brodie MJ, Buchhalter J, Cross JH, van Emde Boas W, Engel J, French J, Glauser TA, Mathern GW, et al. 2010. Revised terminology and concepts for organization of seizures and epilepsies: Report of the ILAE commission on classification and terminology, 2005-2009. Epilepsia 51(4):676-85.

Bialer M. 2011. Chemical properties of antiepileptic drugs (AEDs). Adv Drug Deliv Rev 64(10):887-95.

Bien CG, Urbach H, Schramm J, Soeder BM, Becker AJ, Voltz R, Vincent A, Elger CE. 2007. Limbic encephalitis as a precipitating event in adult-onset temporal lobe epilepsy. Neurology 69(12):1236-44.

Blanc F, Martinian L, Liagkouras I, Catarino C, Sisodiya SM, Thom M. 2011. Investigation of widespread neocortical pathology associated with hippocampal sclerosis in epilepsy: A postmortem study. Epilepsia 52(1):10-21.

Blümcke I, Thom M, Aronica E, Armstrong DD, Bartolomei F, Bernasconi A, Bernasconi N, Bien CG, Cendes F, Coras R, et al. 2013. International consensus classification of hippocampal sclerosis in temporal lobe epilepsy: A task force report from the ILAE commission on diagnostic methods. Epilepsia 54(7):1315-29.

Blumenfeld H, McNally KA, Vanderhill SD, Paige AL, Chung R, Davis K, Norden AD, Stokking R, Studholme C, Novotny EJ,Jr, et al. 2004. Positive and negative network correlations in temporal lobe epilepsy. Cereb Cortex 14(8):892-902.

Briellmann RS, Berkovic SF, Syngeniotis A, King MA, Jackson GD. 2002. Seizure-associated hippocampal volume loss: A longitudinal magnetic resonance study of temporal lobe epilepsy. Ann Neurol 51(5):641-4.

Butler T, Li Y, Tsui W, Friedman D, Maoz A, Wang X, Harvey P, Tanzi E, Morim S, Kang Y, et al. 2016. Transient and chronic seizure-induced inflammation in human focal epilepsy. Epilepsia 57(9):e191-4.

Butt AN and Swaminathan R. 2008. Overview of circulating nucleic acids in plasma/serum. Ann N Y Acad Sci 1137:236-42.

Callaghan BC, Anand K, Hesdorffer D, Hauser WA, French JA. 2007. Likelihood of seizure remission in an adult population with refractory epilepsy. Ann Neurol 62(4):382-9.

Campello Yurgel V, Ikuta N, Brondani da Rocha A, Lunge VR, Fett Schneider R, Kazantzi Fonseca AS, Grivicich I, Zanoni C, Regner A. 2007. Role of plasma DNA as a predictive marker of fatal outcome following severe head injury in males. J Neurotrauma 24(7):1172-81.

Cascino GD. 2002. Video-EEG monitoring in adults. Epilepsia 43 Suppl 3:80-93.

Chang CP, Chia RH, Wu TL, Tsao KC, Sun CF, Wu JT. 2003. Elevated cell-free serum DNA detected in patients with myocardial infarction. Clin Chim Acta 327(1-2):95-101.

Chen W, Tan Y, Ge Y, Chen Y, Liu X. 2015. The effects of levetiracetam on cerebrospinal fluid and plasma NPY and GAL, and on the components of stress response system, hs-CRP, and S100B protein in serum of patients with refractory epilepsy. Cell Biochem Biophys 73(2):489-94.

Claes L, Del-Favero J, Ceulemans B, Lagae L, Van Broeckhoven C, De Jonghe P. 2001. De novo mutations in the sodium-channel gene SCN1A cause severe myoclonic epilepsy of infancy. Am J Hum Genet 68(6):1327-32.

Commins SP, Borish L, Steinke JW. 2010. Immunologic messenger molecules: Cytokines, interferons, and chemokines. J Allergy Clin Immunol 125(2 Suppl 2): S53-72.

Cukiert A and Lehtimäki K. 2017. Deep brain stimulation targeting in refractory epilepsy. Epilepsia 58 Suppl 1:80-4.

De Simoni MG, Perego C, Ravizza T, Moneta D, Conti M, Marchesi F, De Luigi A, Garattini S, Vezzani A. 2000. Inflammatory cytokines and related genes are induced in the rat hippocampus by limbic status epilepticus. Eur J Neurosci 12(7):2623-33.

de Vries EE, van den Munckhof B, Braun KP, van Royen-Kerkhof A, de Jager W, Jansen FE. 2016. Inflammatory mediators in human epilepsy: A systematic review and meta-analysis. Neurosci Biobehav Rev 63:177-90.

Delongui F, Lozovoy MAB, Iriyoda TMV, Costa NT, Stadtlober NP, Alfieri DF, Flauzino T, Dichi I, Simao ANC, Reiche EMV. 2017. C-reactive protein +1444CT (rs1130864) genetic polymorphism is associated with the susceptibility to systemic lupus erythematosus and C-reactive protein levels. Clin Rheumatol 36(8):1779-88.

Devinsky O, Hesdorffer DC, Thurman DJ, Lhatoo S, Richerson G. 2016. Sudden unexpected death in epilepsy: Epidemiology, mechanisms, and prevention. Lancet Neurol 15(10):1075-88.

Dixit AB, Tripathi M, Chandra PS, Banerjee J. 2016. Molecular biomarkers in drug-resistant epilepsy: Facts & possibilities. Int J Surg 36(Pt B):483-91.

Dinarello CA. 2009. Immunological and inflammatory functions of the interleukin-1 family. Annu Rev Immunol 27:519-50.

Dinarello CA. 2000. Proinflammatory cytokines. Chest 118(2):503-8.

Dinarello CA. 1996. Biologic basis for interleukin-1 in disease. Blood 87(6):2095-147.

Donato AJ, Black AD, Jablonski KL, Gano LB, Seals DR. 2008. Aging is associated with greater nuclear NFkappaB, reduced IkappaBalpha, and increased expression of proinflammatory cytokines in vascular endothelial cells of healthy humans. Aging Cell 7(6):805-12.

Dubé CM, Ravizza T, Hamamura M, Zha Q, Keebaugh A, Fok K, Andres AL, Nalcioglu O, Obenaus A, Vezzani A, et al. 2010. Epileptogenesis provoked by prolonged experimental febrile seizures: Mechanisms and biomarkers. J Neurosci 30(22):7484-94.

Dziedzic T. 2006. Systemic inflammatory markers and risk of dementia. Am J Alzheimers Dis Other Demen 21(4):258-62.

El-Khatib F, Rauchenzauner M, Lechleitner M, Hoppichler F, Naser A, Waldmann M, Trinka E, Unterberger I, Bauer G, Luef GJ. 2007. Valproate, weight gain and carbohydrate craving: A gender study. Seizure 16(3):226-32.

Elkind MS, Luna JM, Moon YP, Liu KM, Spitalnik SL, Paik MC, Sacco RL. 2009. High-sensitivity C-reactive protein predicts mortality but not stroke: The northern manhattan study. Neurology 73(16):1300-7.

Endele S, Rosenberger G, Geider K, Popp B, Tamer C, Stefanova I et al. 2010. Mutations in *GRIN2A* and *GRIN2B* encoding regulatory subunits of NMDA receptors cause variable neurodevelopmental phenotypes. Nat Genet 42:1021-26.

Engel J,Jr and International League Against Epilepsy (ILAE). 2001. A proposed diagnostic scheme for people with epileptic seizures and with epilepsy: Report of the ILAE task force on classification and terminology. Epilepsia 42(6):796-803.

Engelhart MJ, Geerlings MI, Meijer J, Kiliaan A, Ruitenberg A, van Swieten JC, Stijnen T, Hofman A, Witteman JC, Breteler MM. 2004. Inflammatory proteins in plasma and the risk of dementia: The rotterdam study. Arch Neurol 61(5):668-72.

Englot DJ, Yang L, Hamid H, Danielson N, Bai X, Marfeo A, Yu L, Gordon A, Purcaro MJ, Motelow JE, et al. 2010. Impaired consciousness in temporal lobe seizures: Role of cortical slow activity. Brain 133(Pt 12):3764-77.

Esch T and Stefano G. 2002. Proinflammation: A common denominator or initiator of different pathophysiological disease processes. Med Sci Monit 8(5): HY1-9.

Esen Melez I, Arslan MN, Melez DO, Sanli AN, Koc S. 2017. Sudden unexpected death in epilepsy: A retrospective autopsy study of 112 epileptic patients. Noro Psikiyatr Ars 54(3):225-33.

Fabene PF, Navarro Mora G, Martinello M, Rossi B, Merigo F, Ottoboni L, Bach S, Angiari S, Benati D, Chakir A, et al. 2008. A role for leukocyte-endothelial adhesion mechanisms in epilepsy. Nat Med 14(12):1377-83.

Fakhoury M. 2015. Role of immunity and inflammation in the pathophysiology of neurodegenerative diseases. Neurodegener Dis 15(2):63-9.

Fang Z, Yang Y, Chen X, Zhang W, Xie Y, Chen Y, Liu Z, Yuan W. 2017. Advances in autoimmune epilepsy associated with antibodies, their potential pathogenic molecular mechanisms, and current recommended immunotherapies. Front Immunol 8:395.

Farhang A, Javanmard SH, Mehvari J, Zare M, Saadatnia M. 2016. Inflammation and endothelium response in epileptic patients: A case-control study. Adv Biomed Res 5:131,9175.187370. eCollection 2016.

Fiala M, Avagyan H, Merino JJ, Bernas M, Valdivia J, Espinosa-Jeffrey A, Witte M, Weinand M. 2013. Chemotactic and mitogenic stimuli of neuronal apoptosis in patients with medically intractable temporal lobe epilepsy. Pathophysiology 20:59–69.

Fiest KM, Sauro KM, Wiebe S, Patten SB, Kwon CS, Dykeman J, Pringsheim T, Lorenzetti DL, Jette N. 2017. Prevalence and incidence of epilepsy: A systematic review and meta-analysis of international studies. Neurology 88(3):296-303.

Fisher RS. 2017. An overview of the 2017 ILAE operational classification of seizure types. Epilepsy Behav 70(PtA):271-3.

Fisher RS, van Emde Boas W, Blume W, Elger C, Genton P, Lee P, Engel J, Jr. 2005. Epileptic seizures and epilepsy: Definitions proposed by the international league against epilepsy (ILAE) and the international bureau for epilepsy (IBE). Epilepsia 46(4):470-2.

Fisher RS, Cross JH, French JA, Higurashi N, Hirsch E, Jansen FE, Lagae L, Moshé SL, Peltola J, Roulet Perez E, et al. 2017a. Operational classification of seizure types by the international league against epilepsy: Position paper of the ILAE commission for classification and terminology. Epilepsia 58(4):522-30.

Fisher RS, Cross JH, D'Souza C, French JA, Haut SR, Higurashi N, Hirsch E, Jansen FE, Lagae L, Moshe SL, et al. 2017b. Instruction manual for the ILAE 2017 operational classification of seizure types. Epilepsia 58(4):531-42.

Fisher RS, Acevedo C, Arzimanoglou A, Bogacz A, Cross JH, Elger CE, Engel J,Jr, Forsgren L, French JA, Glynn M, et al. 2014. ILAE official report: A practical clinical definition of epilepsy. Epilepsia 55(4):475-82.

Gaily E, Lommi M, Lapatto R, Lehesjoki AE. 2016. Incidence and outcome of epilepsy syndromes with onset in the first year of life: A retrospective population-based study. Epilepsia 57(10):1594-601.

Gales JM and Prayson RA. 2017. Chronic inflammation in refractory hippocampal sclerosis-related temporal lobe epilepsy. Ann Diagn Pathol 30:12-6.

Gales JM, Jehi L, Nowacki A, Prayson RA. 2017. The role of histopathologic subtype in the setting of hippocampal sclerosis-associated mesial temporal lobe epilepsy. Hum Pathol 63:79-88.

Gallentine WB, Shinnar S, Hesdorffer DC, Epstein L, Nordli DR, Jr, Lewis DV, Frank LM, Seinfeld S, Shinnar RC, Cornett K, et al. 2017. Plasma cytokines associated with febrile status epilepticus in children: A potential biomarker for acute hippocampal injury. Epilepsia 58(6):1102-1111.

Gerlach H. 2016. Agents to reduce cytokine storm. F1000res 5:2909.

Harward SC, Chen WC, Rolston JD, Haglund MM, Englot DJ. 2017. Seizure outcomes in occipital lobe and posterior quadrant epilepsy surgery: A systematic review and meta-analysis. Neurosurgery, nyx158.

He JJ, Sun FJ, Wang Y, Luo XQ, Lei P, Zhou J, Zhu D, Li ZY, Yang H. 2016. Increased expression of interleukin 17 in the cortex and hippocampus from patients with mesial temporal lobe epilepsy. J Neuroimmunol 298:153-9.

Heida JG, Moshé SL, Pittman QJ. 2009. The role of interleukin-1beta in febrile seizures. Brain Dev 31(5):388-93.

Heinrich PC, Behrmann I, Haan S, Hermanns HM, Muller-Newen G, Schaper F. 2003. Principles of interleukin (IL)-6-type cytokine signalling and its regulation. Biochem J 374(Pt 1):1-20.

Herder C, Peltonen M, Koenig W, Sutfels K, Lindstrom J, Martin S, Ilanne-Parikka P, Eriksson JG, Aunola S, Keinanen-Kiukaanniemi S, et al. 2009. Anti-inflammatory effect of lifestyle changes in the finnish diabetes prevention study. Diabetologia 52(3):433-42.

Hermann BP, Sager MA, Koscik RL, Young K, Nakamura K. 2017. Vascular, inflammatory, and metabolic factors associated with cognition in aging persons with chronic epilepsy. Epilepsia 58(11):e152-6.

Hesdorffer DC, Benn EK, Cascino GD, Hauser WA. 2009. Is a first acute symptomatic seizure epilepsy? mortality and risk for recurrent seizure. Epilepsia 50(5):1102-8.

Hesdorffer DC, Logroscino G, Benn EK, Katri N, Cascino G, Hauser WA. 2011. Estimating risk for developing epilepsy: A population-based study in rochester, minnesota. Neurology 76(1):23-7.

Hesdorffer DC, Tomson T, Benn E, Sander JW, Nilsson L, Langan Y, Walczak TS, Beghi E, Brodie MJ, Hauser WA, et al. 2012. Do antiepileptic drugs or generalized tonic-clonic seizure frequency increase SUDEP risk? A combined analysis. Epilepsia 53(2):249-52.

Holst AG, Winkel BG, Risgaard B, Nielsen JB, Rasmussen PV, Haunso S, Sabers A, Uldall P, Tfelt-Hansen J. 2013. Epilepsy and risk of death and sudden unexpected death in the young: A nationwide study. Epilepsia 54(9):1613-20.

Holtman L, van Vliet EA, Aronica E, Wouters D, Wadman WJ, Gorter JA. 2013. Blood plasma inflammation markers during epileptogenesis in post-status epilepticus rat model for temporal lobe epilepsy. Epilepsia 54(4):589-95.

Hulkkonen J, Koskikallio E, Rainesalo S, Keränen T, Hurme M, Peltola J. 2004. The balance of inhibitory and excitatory cytokines is differently regulated in vivo and in vitro among therapy resistant epilepsy patients. Epilepsy Res 59(2-3):199-205.

Hung J, Knuiman MW, Divitini ML, Davis T, Beilby JP. 2008. Prevalence and risk factor correlates of elevated C-reactive protein in an adult australian population. Am J Cardiol 101(2):193-8.

Huttunen R, Kuparinen T, Jylhävä J, Aittoniemi J, Vuento R, Huhtala H, Laine J, Syrjänen J, Hurme M. 2011. Fatal outcome in bacteremia is characterized by high plasma cell free DNA concentration and apoptotic DNA fragmentation: a prospective cohort study. PloS One 6(7): e21700.

Ip Q, Malone DC, Chong J, Harris RB, Labiner DM. 2017. An update on the prevalence and incidence of epilepsy among older adults. Epilepsy Res 139:107-12.

Irwin MR and Cole SW. 2011. Reciprocal regulation of the neural and innate immune systems. Nat Rev Immunol 11(9):625-32.

Ishikawa N, Kobayashi Y, Fujii Y, Kobayashi M. 2015. Increased interleukin-6 and high-sensitivity C-reactive protein levels in pediatric epilepsy patients with frequent, refractory generalized motor seizures. Seizure 25:136-40.

Jadhav T and Cross JH. 2012. Surgical approaches to treating epilepsy in children. Curr Treat Options Neurol 14(6):620-9.

Jefferson AL, Massaro JM, Wolf PA, Seshadri S, Au R, Vasan RS, Larson MG, Meigs JB, Keaney JF, Jr, Lipinska I, et al. 2007. Inflammatory biomarkers are associated with total brain volume: The Framingham heart study. Neurology 68(13):1032-8.

Jung K, Fleischhacker M, Rabien A. 2010. Cell-free DNA in the blood as a solid tumor biomarker--a critical appraisal of the literature. Clin Chim Acta 411(21-22):1611-24.

Jylhävä J, Jylhä M, Lehtimäki T, Hervonen A, Hurme M. 2012. Circulating cell-free DNA is associated with mortality and inflammatory markers in nonagenarians: The vitality 90+ study. Exp Gerontol 47(5):372-8.

Jylhävä J, Nevalainen T, Marttila S, Jylhä M, Hervonen A, Hurme M. 2013. Characterization of the role of distinct plasma cell-free DNA species in age-associated inflammation and frailty. Aging Cell 12(3):388-97.

Jylhävä J, Lyytikainen LP, Kahonen M, Hutri-Kahonen N, Kettunen J, Viikari J, Raitakari OT, Lehtimäki T, Hurme M. 2012. A genome-wide association study identifies UGT1A1 as a regulator of serum cell-free DNA in young adults: The cardiovascular risk in young finns study. PLoS One 7(4):e35426.

Kalueff AV, Lehtimäki KA, Ylinen A, Honkaniemi J, Peltola J. 2004. Intranasal administration of human IL-6 increases the severity of chemically induced seizures in rats. Neurosci Lett 365(2):106-10.

Kan AA, de Jager W, de Wit M, Heijnen C, van Zuiden M, Ferrier C et al. 2012. Protein expression profiling of inflammatory mediators in human temporal lobe epilepsy reveals co-activation of multiple chemokines and cytokines. J Neuroinflammation 9:207.

Kanemoto K, Kawasaki J, Yuasa S, Kumaki T, Tomohiro O, Kaji R, Nishimura M. 2003. Increased frequency of interleukin-1beta-511T allele in patients with temporal lobe epilepsy, hippocampal sclerosis, and prolonged febrile convulsion. Epilepsia 44(6):796-9.

Kempuraj D, Thangavel R, Selvakumar GP, Zaheer S, Ahmed ME, Raikwar SP, Zahoor H, Saeed D, Natteru PA, Iyer S, et al. 2017. Brain and peripheral atypical inflammatory mediators potentiate neuroinflammation and neurodegeneration. Front Cell Neurosci 11:216.

Koskinen JO, Vaarno J, Meltola NJ, Soini JT, Hanninen PE, Luotola J, Waris ME, Soini AE. 2004. Fluorescent nanoparticles as labels for immunometric assay of C-reactive protein using two-photon excitation assay technology. Anal Biochem 328(2):210-8.

Kushner, I, Rzewnicki D, Samols D. 2006. What does minor elevation of C-reactive protein signify? Am J Med 119(2): 166.e17,166. e28.

Kwak A, Lee Y, Kim H, Kim S. 2016. Intracellular interleukin (IL)-1 family cytokine processing enzyme. Arch Pharm Res 39(11):1556-64.

Kwan P and Brodie MJ. 2010. Definition of refractory epilepsy: Defining the indefinable? Lancet Neurol 9(1):27-9.

Kwan P and Brodie MJ. 2000. Early identification of refractory epilepsy. N Engl J Med 342(5):314-9.

Kwan P, Arzimanoglou A, Berg AT, Brodie MJ, Allen Hauser W, Mathern G, Moshé SL, Perucca E, Wiebe S, French J. 2010. Definition of drug resistant epilepsy: Consensus proposal by the ad hoc task force of the ILAE commission on therapeutic strategies. Epilepsia 51(6):1069-77.

Lahat E, Livne M, Barr J, Katz Y. 1997. Interleukin-1beta levels in serum and cerebrospinal fluid of children with febrile seizures. Pediatr Neurol 17(1):34-6.

Laihia JK, Koskinen JO, Waris ME, Jansen CT. 2005. Adaptation of the human skin by chronic solar-simulating UV irradiation prevents ultraviolet-B irradiation-induced rise in serum C-reactive protein levels. Photochem Photobiol 81(3):654-8.

Lee RW and Worrell GA. 2012. Dorsolateral frontal lobe epilepsy. J Clin Neurophysiol 29(5):379-84.

Lehtimäki KA, Liimatainen S, Peltola J, Arvio M. 2011. The serum level of interleukin-6 in patients with intellectual disability and refractory epilepsy. Epilepsy Res 95(1-2):184-7.

Lehtimäki KA, Keränen T, Palmio J, Peltola J. 2010. Levels of IL-1beta and IL-1ra in cerebrospinal fluid of human patients after single and prolonged seizures. Neuroimmunomodulation 17(1):19-22.

Lehtimäki KA, Peltola J, Koskikallio E, Keränen T, Honkaniemi J. 2003. Expression of cytokines and cytokine receptors in the rat brain after kainic acid-induced seizures. Brain Res Mol Brain Res 110(2):253-60.

Lehtimäki KA, Keränen T, Palmio J, Mäkinen R, Hurme M, Honkaniemi J, Peltola J. 2007. Increased plasma levels of cytokines after seizures in localization-related epilepsy. Acta Neurol Scand 116(4):226-30.

Lehtimäki KA, Keränen T, Huhtala H, Hurme M, Ollikainen J, Honkaniemi J, Palmio J, Peltola J. 2004. Regulation of IL-6 system in cerebrospinal fluid and serum compartments by seizures: The effect of seizure type and duration. J Neuroimmunol 152(1-2):121-5.

Lhatoo SD, Nei M, Raghavan M, Sperling M, Zonjy B, Lacuey N, Devinsky O. 2016. Nonseizure SUDEP: Sudden unexpected death in epilepsy without preceding epileptic seizures. Epilepsia 57(7):1161-8.

Licastro F, Chiappelli M, Ruscica M, Carnelli V, Corsi MM. 2005. Altered cytokine and acute phase response protein levels in the blood of children with downs syndrome: Relationship with dementia of Alzheimer's type. Int J Immunopathol Pharmacol 18(1):165-72.

Liguori C, Romigi A, Izzi F, Placidi F, Nuccetelli M, Cordella A, Bernardini S, Biagio MN. 2017. Complement system dysregulation in patients affected by idiopathic

generalized epilepsy and the effect of antiepileptic treatment. Epilepsy Res 137:107-11.

Liimatainen S, Fallah M, Kharazmi E, Peltola M, Peltola J. 2009a. Interleukin-6 levels are increased in temporal lobe epilepsy but not in extra-temporal lobe epilepsy. J Neurol 256(5):796-802.

Liimatainen S, Peltola M, Fallah M, Kharazmi E, Haapala AM, Peltola J. 2009b. The high prevalence of antiphospholipid antibodies in refractory focal epilepsy is related to recurrent seizures. Eur J Neurol 16(1):134-41.

Liimatainen S, Peltola M, Sabater L, Fallah M, Kharazmi E, Haapala AM, Dastidar P, Knip M, Saiz A, Peltola J. 2010. Clinical significance of glutamic acid decarboxylase antibodies in patients with epilepsy. Epilepsia 51(5):760-7.

Liimatainen SP, Jylhävä J, Raitanen J, Peltola JT, Hurme MA. 2013. The concentration of cell-free DNA in focal epilepsy. Epilepsy Res 105(3):292-8.

Lin HB, Yang XM, Li TJ, Cheng YF, Zhang HT, Xu JP. 2009. Memory deficits and neurochemical changes induced by C-reactive protein in rats: Implication in Alzheimer's disease. Psychopharmacology (Berl) 204(4):705-14.

Lin Z, Si Q, Xiaoyi Z. 2016. Association between epilepsy and systemic autoimmune diseases: A meta-analysis. Seizure 41:160-6.

Lippi G, Sanchis-Gomar F, Cervellin G. 2015. Cell-free DNA for diagnosing myocardial infarction: Not ready for prime time. Clin Chem Lab Med 53(12):1895-901.

Liu S, Yu W, Lu Y. 2016. The causes of new-onset epilepsy and seizures in the elderly. Neuropsychiatr Dis Treat 12:1425-34.

Malter MP, Frisch C, Zeitler H, Surges R, Urbach H, Helmstaedter C, Elger CE, Bien CG. 2015. Treatment of immune-mediated temporal lobe epilepsy with GAD antibodies. Seizure 30:57-63.

Mantovani A, Garlanda C, Doni A, Bottazzi B. 2008. Pentraxins in innate immunity: From C-reactive protein to the long pentraxin PTX3. J Clin Immunol 28(1):1-13.

Marchi N, Granata T, Ghosh C, Janigro D. 2012. Blood-brain barrier dysfunction and epilepsy: Pathophysiologic role and therapeutic approaches. Epilepsia 53(11):1877-86.

Marchi N, Granata T, Freri E, Ciusani E, Ragona F, Puvenna E, et al. 2011. Efficacy of anti-inflammatory therapy in a model of acute seizures and in a population of pediatric drug resistant epileptics. PLoS One 6(3):e18200.

Marchi N, Fan Q, Ghosh C, fazio V, Bertolini F, Betto G et al. 2009. Antagonism of peripheral inflammation reduces the severity of status epilepticus. Neurobiol Dis. 33(2):171–81.

Marnell L, Mold C, Du Clos TW. 2005. C-reactive protein: Ligands, receptors and role in inflammation. Clin Immunol 117(2):104-11.

Mathern G, Pretorius J, Leite J, Kornblum H, Mendoza D, Lozada A, Bertram III EH. 1998. Hippocampal AMPA and NMDA mRNA levels and subunit immunoreactivity in human temporal lobe epilepsy patients and a rodent model of chronic mesial limbic epilepsy, Epilepsy Res. 32:154-171.

McKee CA and Lukens JR. 2016. Emerging roles for the immune system in traumatic brain injury. Front Immunol 7:556.

Michalak Z, Obari D, Ellis M, Thom M, Sisodiya SM. 2017. Neuropathology of SUDEP: Role of inflammation, blood-brain barrier impairment, and hypoxia. Neurology 88(6):551-61.

Mintzer S, Miller R, Shah K, Chervoneva I, Nei M, Skidmore C, Sperling MR. 2016. Long-term effect of antiepileptic drug switch on serum lipids and C-reactive protein. Epilepsy Behav 58:127-32.

Mintzer S, Skidmore CT, Rankin SJ, Chervoneva I, Pequinot E, Capuzzi DM, Sperling MR. 2012. Conversion from enzyme-inducing antiepileptic drugs to topiramate: Effects on lipids and C-reactive protein. Epilepsy Res 98(1):88-93.

Mintzer S, Skidmore CT, Abidin CJ, Morales MC, Chervoneva I, Capuzzi DM, Sperling MR. 2009. Effects of antiepileptic drugs on lipids, homocysteine, and C-reactive protein. Ann Neurol 65(4):448-56.

Misra UK, Tan CT, Kalita J. 2008. Viral encephalitis and epilepsy. Epilepsia 49 Suppl 6:13-8.

Mittra I, Nair NK, Mishra PK. 2012a. Nucleic acids in circulation: Are they harmful to the host? J Biosci 37(2):301-12.

Mittra I, Nair NK, Mishra PK. 2012b. Nucleic acids in circulation: Are they harmful to the host? J Biosci 37(2):301-12.

Mohanraj R and Brodie MJ. 2006. Diagnosing refractory epilepsy: Response to sequential treatment schedules. Eur J Neurol 13(3):277-82.

Morganti-Kossmann MC, Satgunaseelan L, Bye N, Kossmann T. 2007. Modulation of immune response by head injury. Injury 38(12):1392-400.

Moshé SL, Perucca E, Ryvlin P, Tomson T. 2015. Epilepsy: New advances. Lancet 385(9971):884-98.

Muller-Newen G, Kuster A, Hemmann U, Keul R, Horsten U, Martens A, Graeve L, Wijdenes J, Heinrich PC. 1998. Soluble IL-6 receptor potentiates the antagonistic activity of soluble gp130 on IL-6 responses. J Immunol 161(11):6347-55.

Nevalainen O, Auvinen A, Ansakorpi H, Artama M, Raitanen J, Isojarvi J. 2012. Mortality by clinical characteristics in a tertiary care cohort of adult patients with chronic epilepsy. Epilepsia 53(12):e212-4.

Ngugi AK, Bottomley C, Kleinschmidt I, Sander JW, Newton CR. 2010. Estimation of the burden of active and life-time epilepsy: A meta-analytic approach. Epilepsia 51(5):883-90.

Nouvenne A, Ticinesi A, Lauretani F, Maggio M, Lippi G, Prati B, Borghi L, Meschi P. 2016.The prognostic value of high-sensitivity C-reactive protein and prealbumin for short-term mortality in acutely hospitalized multimorbid elderly patients: a prospective cohort study. J Nutr Health Aging. 20(4):462-8.

Nowak M, Bauer S, Haag A, Cepok S, Todorova-Rudolph A, Tackenberg B, Norwood B, Oertel WH, Rosenow F, Hemmer B, et al. 2011. Interictal alterations of cytokines and leukocytes in patients with active epilepsy. Brain Behav Immun 25(3):423-8.

Nune G, DeGiorgio C, Heck C. 2015. Neuromodulation in the treatment of epilepsy. Curr Treat Options Neurol 17:43.

Ong MS, Kohane IS, Cai T, Gorman MP, Mandl KD. 2014. Population-level evidence for an autoimmune etiology of epilepsy. JAMA Neurol 71(5):569-74.

Peltola J, Hurme M, Miettinen A, Keränen T. 1998. Elevated levels of interleukin-6 may occur in cerebrospinal fluid from patients with recent epileptic seizures. Epilepsy Res 31(2):129-33.

Peltola J, Laaksonen J, Haapala AM, Hurme M, Rainesalo S, Keränen T. 2002. Indicators of inflammation after recent tonic-clonic epileptic seizures correlate with plasma interleukin-6 levels. Seizure 11(1):44-6.

Peltola J, Palmio J, Korhonen L, Suhonen J, Miettinen A, Hurme M, Lindholm D, Keränen T. 2000. Interleukin-6 and interleukin-1 receptor antagonist in cerebrospinal fluid from patients with recent tonic-clonic seizures. Epilepsy Res 41(3):205-11.

Penkowa M, Molinero A, Carrasco J, Hidalgo J. 2001. Interleukin-6 deficiency reduces the brain inflammatory response and increases oxidative stress and neurodegeneration after kainic acid-induced seizures. Neuroscience 102(4):805-18.

Pitkänen A and Sutula TP. 2002. Is epilepsy a progressive disorder? prospects for new therapeutic approaches in temporal-lobe epilepsy. Lancet Neurol 1(3):173-81.

Pitkänen A and Engel J,Jr. 2014. Past and present definitions of epileptogenesis and its biomarkers. Neurotherapeutics 11(2):231-41.

Rainer TH, Wong LK, Lam W, Yuen E, Lam NY, Metreweli C, Lo YM. 2003. Prognostic use of circulating plasma nucleic acid concentrations in patients with acute stroke. Clin Chem 49(4):562-9.

Ravizza T, Terrone G, Salamone A, Frigerio F, Balosso S, Antoine DJ, Vezzani A. 2017. High mobility group box 1 is a novel pathogenic factor and a mechanistic biomarker for epilepsy. Brain Behav Immun pii: S0889-1591(17)30464-6.

Ravizza T, Noe F, Zardoni D, Vaghi V, Sifringer M, Vezzani A. 2008a. Interleukin converting enzyme inhibition impairs kindling epileptogenesis in rats by blocking astrocytic IL-1beta production. Neurobiol Dis 31(3):327-33.

Ravizza T, Gagliardi B, Noe F, Boer K, Aronica E, Vezzani A. 2008b. Innate and adaptive immunity during epileptogenesis and spontaneous seizures: Evidence from experimental models and human temporal lobe epilepsy. Neurobiol Dis 29(1):142-60.

Ristić AJ, Alexopoulos AV, So N, Wong C, Najm IM. 2012. Parietal lobe epilepsy: The great imitator among focal epilepsies. Epileptic Disord 14(1):22-31.

Roseti C, van Vliet EA, Cifelli P, Ruffolo G, Baayen JC, Di Castro MA, Bertollini C, Limatola C, Aronica E, Vezzani A, et al. 2015. GABAA currents are decreased by IL-1beta in epileptogenic tissue of patients with temporal lobe epilepsy: Implications for ictogenesis. Neurobiol Dis 82:311-20.

Rothaug M, Becker-Pauly C, Rose-John S. 2016. The role of interleukin-6 signaling in nervous tissue. Biochim Biophys Acta 1863(6 Pt A):1218-27.

Ryvlin P, Nashef L, Lhatoo SD, Bateman LM, Bird J, Bleasel A, Boon P, Crespel A, Dworetzky BA, Høgenhaven H, et al. 2013. Incidence and mechanisms of cardiorespiratory arrests in epilepsy monitoring units (MORTEMUS): A retrospective study. Lancet Neurol 12(10):966-77.

Sakuma H, Tanuma N, Kuki I, takahashi Y, Shiomi M, Hayashi M. 2014. Intrathecal overproduction of proinflammatory cytokines and chemokines in febrile infection-related refractory status epilepticus. J Neurol Neurosurg Psychiatry 86(7):820–2.

Samland H, Huitron-Resendiz S, Masliah E, Criado J, Henriksen SJ, Campbell IL. 2003. Profound increase in sensitivity to glutamatergic- but not cholinergic agonist-induced seizures in transgenic mice with astrocyte production of IL-6. J Neurosci Res 73(2):176-87.

Saukkonen K, Lakkisto P, Pettila V, Varpula M, Karlsson S, Ruokonen E, Pulkki K, Finnsepsis Study Group. 2008. Cell-free plasma DNA as a predictor of outcome in severe sepsis and septic shock. Clin Chem 54(6):1000-7.

Scheffer IE, Berkovic S, Capovilla G, Connolly MB, French J, Guilhoto L, Hirsch E, Jain S, Mathern GW, Moshe SL, et al. 2017. ILAE classification of the epilepsies: Position paper of the ILAE commission for classification and terminology. Epilepsia 58(4):512-21.

Scheller J, Chalaris A, Schmidt-Arras D, Rose-John S. 2011. The pro- and anti-inflammatory properties of the cytokine interleukin-6. Biochim Biophys Acta 1813(5):878-88.

Schmidt D and Loscher W. 2005. Drug resistance in epilepsy: Putative neurobiologic and clinical mechanisms. Epilepsia 46(6):858-77.

Schmidt R, Schmidt H, Curb JD, Masaki K, White LR, Launer LJ. 2002. Early inflammation and dementia: A 25-year follow-up of the honolulu-asia aging study. Ann Neurol 52(2):168-74.

Schuele SU and Lüders HO. 2008. Intractable epilepsy: Management and therapeutic alternatives. Lancet Neurol 7(6):514-24.

Semple BD, O'Brien TJ, Gimlin K, Wright DK, Kim SE, Casillas-Espinosa PM, Webster KM, Petrou S, Noble-Haeusslein LJ. 2017. Interleukin-1 receptor in seizure susceptibility after traumatic injury to the pediatric brain. J Neurosci 37(33):7864-77.

Shorvon S and Luciano AL. 2007. Prognosis of chronic and newly diagnosed epilepsy: Revisiting temporal aspects. Curr Opin Neurol 20(2):208-12.

Sillanpää M, Jalava M, Shinnar S. 1999. Epilepsy syndromes in patients with childhood-onset seizures in finland. Pediatr Neurol 21(2):533-7.

Sims JE and Smith DE. 2010. The IL-1 family: Regulators of immunity. Nat Rev Immunol 10(2):89-102.

Skidmore CT. 2016. Adult focal epilepsies. Continuum (Minneap Minn) 22(1 Epilepsy):94-115.

Skoch J, Adelson PD, Bhatia S, Greiner HM, Rydenhag B, Scavarda D, Mangano FT. 2017. Subdural grid and depth electrode monitoring in pediatric patients. Epilepsia 58 Suppl 1:56-65.

Sonmez FM, Serin HM, Alver A, Aliyazicioglu R, Cansu A, Can G, Zaman D. 2013. Blood levels of cytokines in children with idiopathic partial and generalized epilepsy. Seizure 22(7):517-21.

Sotgiu S, Murrighile MR, Constantin G. 2010. Treatment of refractory epilepsy with natalizumab in a patient with multiple sclerosis. Case report. BMC Neurology 10:84.

Soydas T, Karaman O, Arkan H, Yenmis G, Ilhan MM, Tombulturk K, Tasan E, Kanigur Sultuybek G. 2016. The correlation of increased CRP levels with NFKB1 and TLR2 polymorphisms in the case of morbid obesity. Scand J Immunol 84(5):278-83.

Spatola M and Dalmau J. 2017. Seizures and risk of epilepsy in autoimmune and other inflammatory encephalitis. Curr Opin Neurol 30(3):345-53.

Spulber S and Schultzberg M. 2010. Connection between inflammatory processes and transmittor function-modulatory effects of interleukin-1. Prog Neurobiol 90(2):256-62.

Strauss KI and Elisevich KV. 2016. Brain region and epilepsy-associated differences in inflammatory mediator levels in medically refractory mesial temporal lobe epilepsy. J Neuroinflammation 13(1):270.

Srivastava A, Dixit AB, Banerjee J, Tripathi M, Sarat Chandra P. 2016. Role of inflammation and its miRNA based regulation in epilepsy: Implications for therapy. Clin Chim Acta 452:1-9.

Talaat FM, Kamel T, Rabah AM, Ahmed AM, El-Jaafary SI, Abdelaziz GH. 2015. Epilepsy and antiepileptic drugs: risk factors for atherosclerosis. Int J Neurosci 125(7):507-11.

Tan TY, Lu CH, Chuang HY, Lin TK, Liou CW, Chang WN, Chuang YC. 2009. Long-term antiepileptic drug therapy contributes to the acceleration of atherosclerosis. Epilepsia 50(6):1579-86.

Tatum WO,4th. 2012. Mesial temporal lobe epilepsy. J Clin Neurophysiol 29(5):356-65.

Thom M. 2014. Review: Hippocampal sclerosis in epilepsy: A neuropathology review. Neuropathol Appl Neurobiol 40(5):520-43.

Thom M, Zhou J, Martinian L, Sisodiya S. 2005. Quantitative post-mortem study of the hippocampus in chronic epilepsy: Seizures do not inevitably cause neuronal loss. Brain 128(Pt 6):1344-57.

Terrone G, Pauletti A, Pascente R, Vezzani A. 2016. Preventing epileptogenesis: A realistic goal? Pharmacol Res 110:96-100.

Toledano M and Pittock SJ. 2015. Autoimmune epilepsy. Semin Neurol 35(3):245-58.

Tsai NW, Lin TK, Chen SD, Chang WN, Wang HC, Yang TM, Lin YJ, Jan CR, Huang CR, Liou CW, et al. 2011. The value of serial plasma nuclear and mitochondrial DNA levels in patients with acute ischemic stroke. Clin Chim Acta 412(5-6):476-9.

Uludag IF, Bilgin S, Zorlu Y, Tuna G, Kirkali G. 2013. Interleukin-6, interleukin-1 beta and interleukin-1 receptor antagonist levels in epileptic seizures. Seizure 22(6):457-61.

Valentine RJ, Vieira VJ, Woods JA, Evans EM. 2009. Stronger relationship between central adiposity and C-reactive protein in older women than men. Menopause 16(1):84-9.

van Vliet EA, da Costa Araujo S, Redeker S, van Schaik R, Aronica E, Gorter JA. 2007. Blood-brain barrier leakage may lead to progression of temporal lobe epilepsy. Brain 130(Pt 2):521-34.

Varadkar S, Bien CG, Kruse CA, Jensen FE, Bauer J, Pardo CA, Vincent A, Mathern GW, Cross JH. 2014. Rasmussen's encephalitis: Clinical features, pathobiology, and treatment advances. Lancet Neurol 13(2):195-205.

Vasilyev FF, Silkov AN, Sennikov SV. 2015. Relationship between interleukin-1 type 1 and 2 receptor gene polymorphisms and the expression level of membrane-bound receptors. Cell Mol Immunol 12(2):222-30.

Venkatesan A and Benavides DR. 2015. Autoimmune encephalitis and its relation to infection. Curr Neurol Neurosci Rep 15(3): 3,015-0529-1.

Vezzani A. 2014. Epilepsy and inflammation in the brain: Overview and pathophysiology. Epilepsy Curr 14(1 Suppl):3-7.

Vezzani A and Viviani B. 2015. Neuromodulatory properties of inflammatory cytokines and their impact on neuronal excitability. Neuropharmacology 96(Pt A):70-82.

Vezzani A, Lang B, Aronica E. 2015a. Immunity and inflammation in epilepsy. Cold Spring Harb Perspect Med 6(2):a022699.

Vezzani A, Dingledine R, Rossetti AO. 2015b. Immunity and inflammation in status epilepticus and its sequelae: Possibilities for therapeutic application. Expert Rev Neurother 15(9):1081-92.

Vezzani A, Balosso S, Ravizza T. 2008. The role of cytokines in the pathophysiology of epilepsy. Brain Behav Immun 22(6):797-803.

Vezzani A, Aronica E, Mazarati A, Pittman QJ. 2013. Epilepsy and brain inflammation. Exp Neurol 244:11-21.

Vezzani A, French J, Bartfai T, Baram TZ. 2011. The role of inflammation in epilepsy. Nat Rev Neurol 7(1):31-40.

Vezzani A, Fujinami RS, White HS, Preux PM, Blumcke I, Sander JW, Loscher W. 2016. Infections, inflammation and epilepsy. Acta Neuropathol 131(2):211-34.

Vezzani A, Conti M, De Luigi A, Ravizza T, Moneta D, Marchesi F, De Simoni MG. 1999. Interleukin-1beta immunoreactivity and microglia are enhanced in the rat hippocampus by focal kainate application: Functional evidence for enhancement of electrographic seizures. J Neurosci 19(12):5054-65.

Vezzani A, Moneta D, Richichi C, Aliprandi M, Burrows SJ, Ravizza T, Perego C, De Simoni MG. 2002. Functional role of inflammatory cytokines and antiinflammatory molecules in seizures and epileptogenesis. Epilepsia 43 Suppl 5:30-5.

Vezzani A, Moneta D, Conti M, Richichi C, Ravizza T, De Luigi A, De Simoni MG, Sperk G, Andell-Jonsson S, Lundkvist J, et al. 2000. Powerful anticonvulsant action of IL-1 receptor antagonist on intracerebral injection and astrocytic overexpression in mice. Proc Natl Acad Sci U S A 97(21):11534-9.

Virta M, Hurme M, Helminen M. 2002. Increased frequency of interleukin-1beta (-511) allele 2 in febrile seizures. Pediatr Neurol 26(3):192-5.

Walker LE, Frigerio F, Ravizza T, Ricci E, Tse K, Jenkins RE, Sills GJ, Jorgensen A, Porcu L, Thippeswamy T, et al. 2017. Molecular isoforms of high-mobility group box 1 are mechanistic biomarkers for epilepsy. J Clin Invest 127(6):2118-32.

Wang S, Jin B, Yang L, Chen C, Ding Y, Guo Y, Wang Z, Ming W, Tang Y, Wang S, et al. 2017. Clinical value and predictors of subclinical seizures in patients with temporal lobe epilepsy undergoing scalp video-EEG monitoring. J Clin Neurosci 44:214-7.

Wang WY, Tan MS, Yu JT, Tan L. 2015. Role of pro-inflammatory cytokines released from microglia in Alzheimer's disease. Ann Transl Med 3(10):136,5839.2015.03.49.

Weber MD, Frank MG, Tracey KJ, Watkins LR, Maier SF. 2015. Stress induces the danger-associated molecular pattern HMGB-1 in the hippocampus of male sprague dawley rats: A priming stimulus of microglia and the NLRP3 inflammasome. J Neurosci 35(1):316-24.

Weckhuysen S, Mandelstam S, Suls A, Audenaert D, Deconinck T, Claes LR, Deprez L, Smets K, Hristova D, Yordanova I, et al. 2012. KCNQ2 encephalopathy: Emerging phenotype of a neonatal epileptic encephalopathy. Ann Neurol 71(1):15-25.

Woernle CM, Neidert MC, Wulf MA, Burkhardt JK, Grunwald T, Bernays RL. 2013. Excessively elevated C-reactive protein after surgery for temporal lobe epilepsy. Clin Neurol Neurosurg 115(8):1245-9.

Wolf J, Rose-John S, Garbers C. 2014. Interleukin-6 and its receptors: A highly regulated and dynamic system. Cytokine 70(1):11-20.

Wolf J, Waetzig GH, Chalaris A, Reinheimer TM, Wege H, Rose-John S, Garbers C. 2016. Different soluble forms of the interleukin-6 family signal transducer gp130 fine-tune the blockade of interleukin-6 trans-signaling. J Biol Chem 291(31):16186-96.

Xia DL, Zhang H, Luo QL, Zhang AF, Zhu LX. 2016a. Cell-free DNA increase over first 48 hours in emergency intensive care unit predicts fatal outcome in patients with shock. J Int Med Res 44(5):1002-12.

Xia DL, Zhang H, Luo QL, Zhang AF, Zhu LX. 2016b. Cell-free DNA increase over first 48 hours in emergency intensive care unit predicts fatal outcome in patients with shock. J Int Med Res 44(5):1002-12.

Yoshinaga, R., Doi, Y., Ayukawa, K., & Ishikawa, S. (2017). High-sensitivity C reactive protein as a predictor of inhospital mortality in patients with cardiovascular disease at an emergency department: a retrospective cohort study. BMJ Open 7(10), e015112.

Yu HM, Liu WH, He XH, Peng BW. 2012. IL-1beta: An important cytokine associated with febrile seizures? Neurosci Bull 28(3):301-8.

Yuen AW, Bell GS, Peacock JL, Koepp MM, Patsalos PN, Sander JW. 2010. Effects of AEDs on biomarkers in people with epilepsy: CRP, HbA1c and eGFR. Epilepsy Res 91(2-3):187-92.

Zeiler FA, Matuszczak M, Teitelbaum J, Kazina CJ, Gillman LM. 2017. Intravenous immunoglobulins for refractory status epilepticus, part I: A scoping systematic review of the adult literature. Seizure 45:172-80.

Zeiler FA, Matuszczak M, Teitelbaum J, Kazina CJ, Gillman LM. 2016. Plasmapheresis for refractory status epilepticus, part I: A scoping systematic review of the adult literature. Seizure 43:14-22.

Zhao SC, Ma LS, Chu ZH, Xu H, Wu WQ, Liu F. 2017. Regulation of microglial activation in stroke. Acta Pharmacol Sin 38(4):445-58.

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Interleukin-6, interleukin-1 receptor antagonist and interleukin-1beta production in patients with focal epilepsy: A video–EEG study

T. Alapirtti [a,*], S. Rinta [b], J. Hulkkonen [c], R. Mäkinen [d], T. Keränen [a], J. Peltola [a]

[a] Department of Neurosciences and Rehabilitation, Tampere University Hospital, Tampere, Finland
[b] Neuroimmunology Unit, Neurology, Medical School, University of Tampere, Tampere, Finland
[c] Department of Clinical Physiology, Tampere University Hospital, Tampere, Finland
[d] Department of Clinical Neurophysiology, Pirkanmaa Hospital District, Tampere, Finland

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ABSTRACT

Experimental and clinical studies have shown that prolonged seizures result in increased cytokine production in the central nervous system. The purpose of this study was to examine plasma concentrations of interleukin-6 (IL-6), interleukin-1 receptor antagonist (IL-1Ra), and interleukin-1 beta (IL-1β) in 20 patients with epilepsy undergoing a video–EEG study. Plasma samples were obtained at the onset of the recordings and 3, 6, 12 and 24 h after the index seizure. Localization of the seizure focus and classification of epilepsy was based on concordant electroclinical findings in the video–EEG study, and on MRI examination. Patients were divided into two groups: temporal lobe epilepsy (TLE) ($n=11$), and extratemporal lobe epilepsy (XLE) ($n=9$).
Results: Only the TLE group showed significant increase in plasma levels of IL-6 peaking at 6 h postictally. Postictal plasma levels of IL-1RA and IL-1β did not significantly differ from baseline levels in either of the patient groups. IL-1RA showed a decreasing trend ($p>0.059$) in TLE patients during 12 to 24 postictal hours.
Conclusions: This study further supports the role of focal seizures in regulation of cytokine responses.
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1. Introduction

There is ample experimental and clinical evidence demonstrating an increased production of inflammatory cytokines in association with epileptic seizures [1]. In experimental models interleukin-1beta (IL-1β) appears to be proconvulsant and neurotoxic whereas interleukin-1 receptor antagonist (IL-1Ra) is anticonvulsant and neuroprotective [2]. The role of IL-6 is less well defined, but there are some data suggesting that IL-6 acts more as a proconvulsant [3].

In humans, the levels of IL-6 and IL-1Ra were increased after epileptic seizures both in cerebrospinal fluid (CSF) and plasma within 24 h after secondarily generalized tonic–clonic [4,5] and focal seizures [5,6]. In epileptic patients the production of IL-6 is dependent on the extent of spread and duration of the seizure [5]. There were no significant changes in IL-1β concentrations [4,6].These studies demonstrated that both focal and generalized seizures in humans cause changes in IL-6 levels in CSF which are reflected accordingly in peripheral blood.

Accumulating experimental data suggest the importance of specific brain regions and hemispheric lateralization with regard to inflammatory responses in the central nervous system. The effect of injection of exogenous IL-1β was dependent on the site of application with neurodegeneration observed only in the dentate gyrus of the hippocampus but not in the cortex, substantia nigra or striatum [7]. In the kindling model of temporal lobe seizures, kindling on the left but not the right side induced a hyper-responsive immune state [8]. This is consistent with previous data suggesting that under physiological conditions the right neocortex depresses immune functions whereas the left hemisphere enhances them [9].

The purpose of this study is to evaluate cytokine responses in well-characterized patients with focal seizures. video–EEG is an excellent tool for this evaluation since it offers a possibility of exactly defining the extent, localization and duration of seizures in a controlled environment. Therefore we assessed whether the epilepsy or seizure type has an impact on seizure-related production of three cytokines, IL-6, IL-1Ra and IL-1β.

2. Materials and methods

2.1. The classification of patients

Twenty patients with refractory localization-related epilepsy admitted to the video–EEG monitoring unit of the Tampere University Hospital were included in the study. The study was approved by the Ethics Committee of the Tampere University Hospital, and all the patients gave their written informed consent. The study group comprised 7 men and 13 women.

* Corresponding author. Mailing address: Tampere University Hospital, Department of Neurosciences and Rehabilitation, PL 2000, 33521 Tampere, Finland. Tel.: +358 3 3116 5111; fax: +358 3 3116 4351.
E-mail address: tiina.alapirtti@pshp.fi (T. Alapirtti).

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Table 1
Clinical characteristics of patients with temporal epilepsy.

	Epilepsy syndrome/ lateralization	Age (F/M)	Duration of epilepsy years	MRI findings	Seizures/ month during last year	Medication/Treatment	Recorded index seizure type and lateralization	Seizures before index seizure during the previous 24 h	Number of seizures after index seizure during next 24 h
1[a]	TLE/right	36/F	17	Hippocampal sclerosis	13–19	OXC 1200 mg, CZP 1 mg/VNS	CPS right	No	No
2	TLE/right	58/F	42	Hippocampal sclerosis	10–25	CBZ 1400 mg, TGB 45 mg	CPS right	No	No
3[a]	TLE/left	33/F	22	Hippocampal sclerosis	4	LTG 400 mg, TPM 400 mg	CPS left	No	8 CPS left
4[a]	TLE/left	43/M	2	Normal	1	OXC 1500 mg	SGTCS left	No	SGTCS left
5	TLE/left	39/M	12	Normal	15	CBZ 1200 mg, VPA 2500 mg	CPS left	No	No
6[a]	TLE/left	30/M	25	Hippocampal hamartoma	1–4	VPA 1500 mg, LTG 200 mg, TPM 50 mg	CPS left	1 CPS left	1 CPS left
7	TLE/left	52/M	52	Hippocampal sclerosis	4–6	CBZ 600 mg, LEV 2000 mg, LTG 500 mg	CPS left	No	3 CPS left
8	TLE/right	58/F	32	Normal	2–17	CBZ 800 mg, GBP 3600 mg, CZP 1 mg	CPS right	No	4 CPS right
9[a]	TLE/left	28/M	16	Hippocampal sclerosis	1	OXC 600 mg, TPM 500 mg	SGTCS left	No	2 SGTCS left
10[a]	TLE/left	57/F	56	Hippocampal sclerosis	3	CBZ 1000 mg	CPS left	1 CPS left	2 CPS left
11[a]	TLE/unknown	20/F	14	Cortical dysplasia	7–45	OXC 1200 mg, GBP 3600 mg, CLB 10 mg/VNS	SPS unknown	No	2 SPS left

Abbreviations: CBZ, carbamazepine; CLB, clobazam; CZP, clonazepam; CPS, complex partial seizure; GBP, gapapentin; LEV, levetiracetam; LTG, lamotrigine; OXC, oxcarbazepine; TLE, temporal lobe epilepsy; SGTCS, secondary generalized tonic–clonic seizure; SPS, simple partial seizure, TGB, tiagabine; TPM, topiramate; VNS, vagus nerve stimulator; VPA, valproic acid.
[a] IL6 level increase after the index seizure.

All the patients underwent continuous video–EEG monitoring lasting for four days for electroclinical characterization of their seizures as part of routine clinical evaluation for possible epilepsy surgery. Ictal scalp recordings were obtained using synchronous digital video and 22 channel standard bipolar EEG. Electrodes were placed according to the International 10–20 System with additional mastoid and anterior cheek electrodes. All patients underwent a diagnostic brain MRI examination on a 1.5 T machine (General Electric Sigma Easyvision, Wisconsin, USA).

Patients were divided into two groups: those with temporal lobe epilepsy (TLE; Table 1) ($n=11$) and those with extratemporal lobe epilepsy (XLE; Table 2) ($n=9$). The mean ages of the patients in the TLE and XLE groups were 41.3 years (range 20–58) and 28.1 years (range 16–52), respectively. The mean duration of epilepsy in the TLE group was 26.4 years (range 2–52), and in the XLE group it was 18.6 years (range 1–52). The mean seizure frequency per month during the previous year in the TLE group was 9.1 seizures (range 1–45), and in the XLE group 52.5 (range 1–200). The mean duration of the index seizure was 117 s in the XLE group and 69 s in the TLE group. Seizure burden was measured by total duration of seizures recorded during the 24 h sampling period.

Table 2
Clinical characteristics of patients with extratemporal epilepsy.

	Epilepsy syndrome/ lateralization	Age (F/M)	Duration of epilepsy years	MRI findings	Seizures/month during last year	Medication/ Treatment	Recorded index seizure type and lateralization	Seizures before index seizure during the previous 24 h	Number seizures after index seizure during next 24 h
1[a]	PLE/right	26/M	22	Vascular lesion	12–16	LTG 300 mg, LEV 1500 mg	CPS right	3 CPS right	5 CPS right, 1 SGTCS right
2	FLE/right	22/F	3	Normal	1	No	SGTCS right	No	No
3	PLE/left	52/F	52	Cortical dysplasia	35–60	TPM 400 mg, LTG 300 mg	CPS left	No	8 CPS left, 2 SPS left
4	FLE/left and right	16/F	1	Normal	30–40	CBZ 1500 mg, LEV 4000 mg, GBP 2400 mg	CPS right	No	1 CPS left, 1 SGTCS right
5[a]	FLE/right	26/M	9	Vascular lesion	200	CBZ 1500 mg, LEV 4000 mg, GBP 2400 mg	SPS right	1 SPS right	5 SPS right, 1 CPS right
6	FLE/unknown	41/F	31	Normal	>30	PHT 250 mg, CZP 1 mg/VNS	CPS multifocal	No	180 CPS seizures
7	FLE/left	31/F	31	Normal	60–90	LTG 400 mg, CBZ 1600 mg, LEV 1500 mg	CPS left	2 CPS left	1 CPS left
8	FLE/left	17/F	1	Cortical dysplasia	4–20	OXC 1200 mg, TPM 300 mg, LEV 3000 mg	CPS left	3 CPS left	4 SPS left, 1CPS left
9	FLE/right	22/F	17	Cortical dysplasia	30–90	OXC 1500 mg, TPM 500 mg, LEV 2500 mg	CPS right	3 CPS right, 1 SPS right	3 CPS right

Abbreviations: CZP, clonazepam; CPS, complex partial seizure; FLE, frontal lobe epilepsy; GBP, gapapentin; CBZ, carbamazepine; LEV, levetiracetam; LTG, lamotrigine; OXC, oxcarbazepine; PLE, parietal lobe epilepsy; PHT, phenytoin; SGTCS, secondary generalized tonic–clonic seizure; SPS, simple partial seizure, TGB, tiagabine; TPM, topiramate; VNS, vagus nerve stimulator; VPA, valproic acid; VGB, vigabatrin.
[a]IL6 level increased after the index seizure.

Table 3
Circulating concentrations of IL-6, IL-1Ra and IL-1β and IL-1Ra/IL-1β ratio after the onset of seizure in temporal ($n=11$) and extratemporal epilepsy ($n=9$), values are mean ± SD.

	Epilepsy syndrome	0 h	3 h	6 h	12 h	24 h	P(Friedman ANOVA, $df=4$)
IL-6 pg/ml	Temporal	0.127 ± 0.422	0.546 ± 1.46	1.89 ± 2.49	1.55 ± 2.87	0.691 ± 0.919	0.008[a]
	Extratemporal	0	0	0[b]	0.067 ± 0.200	0.411 ± 0.756	0.031[c]
IL-1Ra pg/ml	Temporal	490 ± 461	510 ± 443	532 ± 481	555 ± 368	425 ± 257	0.042[d]
	Extratemporal	313 ± 96.8	325 ± 133	331 ± 81.1	365 ± 133	334 ± 90.2	NS
IL-1β pg/ml	Temporal	1.75 ± 1.51	1.78 ± 1.51	1.40 ± 1.12	2.45 ± 3.67	1.71 ± 1.86	NS
	Extratemporal	1.58 ± 2.18	1.76 ± 2.81	1.66 ± 2.18	1.94 ± 2.46	2.06 ± 2.78	NS
IL-1Ra/IL-1β	Temporal	479 ± 408	467 ± 460	704 ± 689	716 ± 994	947 ± 1046	NS
	Extratemporal	477 ± 5512	650 ± 518	749 ± 724	935 ± 909	659 ± 742	NS[e]

df = degrees of freedom.
[a] Post hoc, Wilcoxon matched pairs test, $df=1$) 0 h<6 h, $p=0.028$, 3 h<6 h, $p=0.028$, 3 h<12 h, $p=0.043$, 6 h>24 h, $p=0.018$.
[b] Mann–Whitney U-test, temporal vs. extratemporal) $p=0.016$.
[c] Post hoc, Wilcoxon matched pairs test, $df=1$) 0, 3, 6 h<24 h, $p=0.109$.
[d] Post hoc, Wilcoxon matched pairs test, $df=1$) 12 h>24 h, $p=0.059$.
[e] Post hoc, Wilcoxon matched pairs test, $df=1$ 3 h<24 h, $p=0.043$.

2.2. IL-6, IL-1Ra and IL-1β measurements

Plasma samples were collected at the beginning of the four-day recordings, and at 3, 6, 12 and 24 h after the index seizure. Only seizures with unequivocally verified localized onset were considered as index seizure. Localization of the seizure focus was recorded, and seizures were categorized as simple partial, complex partial or secondarily generalized seizures. All other seizures after the index seizure during the next 24 h were also registered.

Commercial ELISA kits were used according to the manufacturer's instructions to detect levels of IL-1Ra (Quantikine® Human IL-1RA Immunoassay, R&D Systems, Minneapolis, MN, USA)and IL-6 (PeliKine® Compact Human IL-6, CLB, Amsterdam, The Netherlands). Absorbancies were read with Multiskan MS version 4.0 spectrophotometer at wavelength 450 nm. IL-1β levels were measured by Luminex kit (High sensitivity Human cytokine Lincoplex Kit, St. Missouri, USA) using Bio-Plex suspension array system (Bio-Rad laboratories Hercules, CA, USA). IL-1β data were collected and analyzed using Bio-Plex Manager™ software 4.1 (Bio-Rad Laboratories) and 5-parameter regression formula was used to calculate the sample concentrations from standard curve. The detection limits of the assays were 22 pg/ml for IL-1Ra, 0.4 pg/ml for IL-6, and 0.06 pg/ml for IL-1β. The intra-assay and inter-assay precisions were 6.2% and 6.7% for IL-1RA, 3.11% and 2.16% for IL-1β, and for IL-6 the manufacturer provided an estimate for both parameters between 5 and 10%.

2.3. Statistical analysis

Friedman analysis of variance (ANOVA) with post hoc comparisons (Wilcoxon matched pairs test), Mann–Whitney U-test were used to compare concentrations of circulating IL-6 and IL-1RA. Fisher's exact test was used for comparison of dichotomous variables. Findings were considered statistically significant at P values less than 0.05. Statistical calculations were carried out using Statistica (ver. Win 5.1D, Statsoft Inc., Tulsa, OK, USA).

3. Results

The TLE group showed significant increase in plasma levels of IL-6, peaking at 6 h postictally (Table 3). On the contrary, no change in IL-6 levels was seen in the XLE patients. Postictal plasma levels of IL-1Ra and IL-1β did not significantly differ from the baseline levels in either of the patient groups. IL-1Ra showed a decreasing trend ($p>0.059$) in the TLE patients during 12 to 24 h postictally. No correlation between the production of IL-6 and IL-1Ra and the etiology of epilepsy was found (Data not shown). Concentrations of IL-6 in the individual patients before and after the index seizure are shown in Fig. 1.

There was no major variance in clinical parameters between the TLE and XLE groups which could explain the difference in cytokine production in the patient groups. Two out of 11 patients with TLE and 3 out of 9 patients with XLE experienced secondarily generalized tonic–clonic seizures (a non-significant difference, Fisher's exact test). Increase in the IL-6 levels with this seizure type was observed in both of the TLE patients and 1 out of the 3 XLE patients ($p=$ NS). In patients without secondarily generalized seizures increased IL6 concentration was observed in 5 out of 9 patients with TLE and 1 out of 6 patients with XLE ($p=0.17$). In patients with elevated IL-6 levels the median number of seizures during the 24-hour sampling period was 3 in the TLE group, and 7 in the XLE group. In patients without elevated IL-6 levels the median number of seizures during the corresponding period was 2.5 in the TLE group and 4 in the XLE group. In one of the patients with XLE more than 180 seizures were recorded without any changes in IL-6, IL-1Ra or IL-1β concentrations.

In order to asses the significance of the number, total duration and seizure type (focal vs. secondarily generalized), we perform the following analysis. During the sampling period the mean number of seizures in the TLE group was 3.1 (range 1–9). In the XLE group one patient had 180 seizures during the 24 h; excluding this patient the mean number in this group was 3.3 (range 1–11). The mean seizure burden (seconds ± S.D.) in TLE was 213 ± 209 and in XLE 679 ± 1141. The median was in TLE 140 s and 232 s in XLE. Five patients (two in the TLE and three in the XLE) had a SGTCS during the sampling period. When only maximum concentrations of individual patients during the sampling period was considered, the mean IL-6 concentration in focal seizures was 1.44 pg/ml in TLE group and 0.58 pg/ml in XLE group, and in SGTCS 6.80 pg/ml in TLE and 1.07 pg/ml in XLE. The mean levels of IL-6, IL-1Ra and IL-1β were not significantly increased between the subgroups of patients based on different etiologies of epilepsy.

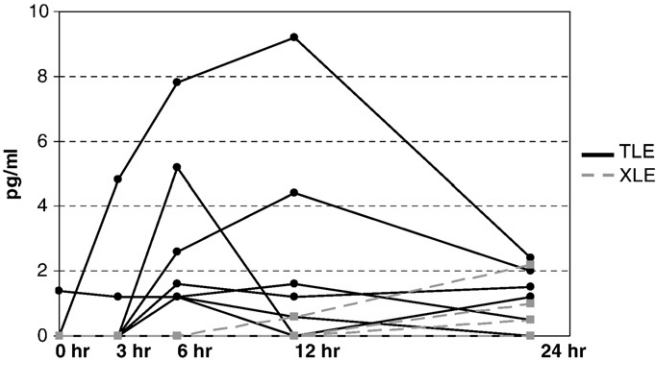

Fig. 1. Concentrations of IL-6 (pg/ml) in the individual patients before (0 h) and after the index seizure.

4. Discussion

Our study is a preliminary report in studying immune functions in focal epilepsies. The major finding in our study was an increased production of IL-6 after focal seizures. The IL-6 concentrations after seizures with temporal lobe onset were significantly elevated both after complex partial and secondarily generalized seizures with peak concentrations at 6 to 12 h postictally, whereas in seizures with extratemporal onset there was only a minor increase in two patients, and no changes were noted after complex partial seizures even in a patient with more than 100 seizures. The mean number or duration of seizures (=seizure burden) or seizure type did not explain the differences between TLE and XLE. Seizures originated in temporal lobes have different extent and pattern of spread compared with those of extratemporal, especially frontal lobe seizures [10]. Temporal lobe seizures probably project to and have effects on the hypothalamus—pituitary axis, which may relate to their immunological responses. However, due to a limited sample size, no definitive conclusions regarding the importance of specific brain region controlling cytokine responses can be made. In future more patients with both TLE and XLE are needed to evaluate the significance of seizure and epilepsy type.

Several experimental studies demonstrate the role of the IL-1 system in seizures. In our study the elevation of IL-1Ra was modest and non-significant, peaking at 12 h both in TLE and XLE. The production of IL-1Ra is mainly activated by IL-1β, so it is possible that upregulation of IL-1Ra reflects the activation of the IL-1 system. On the other hand IL-1β is one of the main inducers of IL-6 [11]. IL-1β is proconvulsant and causes neuronal damage, whereas IL-1Ra counteracts the IL-1 system and it has been suggested that IL-1Ra acts as an anticonvulsive and neuroprotective agent [2]. Although IL-1β did not show a change during the seizure in plasma in our study, it's possible that IL-1β is produced in low quantities locally in the brain and has a role in seizures. IL-1β did not rise after tonic–clonic seizures even in the CSF [4]. IL-1β is upregulated after experimental seizures, but in clinical studies there have not been observable differences in IL-1β concentrations after the seizures [4,12]. This is most likely explained by the findings that the increased expression of IL-1β is observed mainly in the hippocampal area which does not have any effect on the levels of soluble cytokines either in the cerebrospinal fluid or blood circulation.

The significance of the IL-6 activation in epilepsy is less well established. IL-6 is a cytokine known to be rapidly upregulated following different kinds of tissue trauma and inflammation. Experimental results have established an acute temporal release of IL-6 employing a variety of animal models, with detection at 1 h, followed by a peak concentration between 2 and 8 h after injury [13]. IL-6 may act as an activation signal for other cytokines in the brain tissue. These studies also suggest that IL-6 may increase the seizure duration and may cause neurodegeneration. In clinical studies the extent of upregulation in IL-6 levels is determined by seizure spread and duration [5]. There is a clear correlation between cerebrospinal fluid and plasma concentrations of this cytokine, and plasma levels of IL-6 are most likely originated in the central nervous system via venous drainage [4,12].

Temporal lobe epilepsy is at least sometimes a progressive disorder associated with cognitive decline and increasing hippocampal atrophy [14]. Interestingly, IL-1β-mediated neurodegeneration was observed in an experimental study in the dentate gyrus of the hippocampus but not in other brain regions [5]. There is also evidence of generalized brain atrophy as well as cerebellar atrophy in the TLE [15]. In experimental settings, cytokine induction was sometimes observed in the contralateral side and in regions distant from the site of injection [5]. This raises a possibility that cytokine-mediated changes may be responsible for these MRI changes in human patients. There is much less evidence of progressive damage in the extratemporal epilepsy.

Our study and conclusions are confounded by a number of factors. Our results are based on a small patient group. It is, however, difficult to identify and examine a well-defined group of patients with such an information-intensive method as video–EEG. Classification of patients and localization of the seizure focus were based on careful clinical, scalp EEG and radiological assessment, but depth recordings were not available. A different distribution of left- and right-sided foci as well as varying etiologies of epilepsy in our two patient populations could contribute to the variance in the findings between the temporal lobe and extratemporal lobe epilepsies. In future studies larger groups of patients are needed within epileptic syndromes to assess whether the induction of IL-6 and IL-1Ra is associated with various etiological forms of epilepsy such as hippocampal sclerosis, cryptogenic epilepsy or cortical dysplasia. Moreover, it remains to be elucidated whether genetic factors such as genetic polymorphism have an effect on varying kinetics of circulating IL-6, IL-1Ra and IL-1β in TLE and XLE.

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References

[1] Vezzani A, Peltola J, Janigro D. Inflammation in epilepsy. In: Engel P, Pedley T, editors. Epilepsy; a comprehensive textbook. Philadelphia: Lippincott Williams & Wilkins; 2008. p. 267–76.
[2] Vezzani A, Moneta D, Conti M, Richichi C, Ravizza T, De Luigi A, et al. Powerful anticonvulsant action of IL-1 receptor antagonist on intracerebral injection and astrocytic overexpression in mice. Proc Natl Acad Sci U S A 2000;97:11534–9.
[3] Kalueff AV, Lehtimäki KA, Ylinen A, Honkaniemi J, Peltola J. Intranasal administration of human IL-6 increases the severity of chemically induced seizures in rats. Neurosci Lett 2004;269:35–40.
[4] Peltola J, Palmio J, Korhonen L, Suhonen J, Miettinen A, Hurme M, et al. Interleukin-6 and Interleukin-1 receptor antagonist in cerebrospinal fluid. Epilepsy Res 2000;41:205–11.
[5] Lehtimäki KA, Keränen T, Huhtala H, Hurme M, Ollikainen J, Honkaniemi J, et al. Regulation of IL-6 system in cerebrospinal fluid and serum compartments by seizures: the effect of seizure type and duration. J Neuroimmunol 2004;152:121–5.
[6] Lehtimäki K, Keränen T, Palmio J, Mäkinen R, Hurme M, Honkaniemi J, et al. Increased plasma levels of cytokines after seizures in localization-related epilepsy. Acta Neurol Scand 2007;116:226–30.
[7] Depino A, Ferrari C, Pott Godoy MC, Tarelli R, Pitossi FJ. Differential effects of interleukin-1β on neurotoxicity, cytokine induction and glial reaction in spesific brain regions. J Neuroimmunol 2005;168:96–110.
[8] Goldstein KR, Bhatt R, Barton BE, Zalcman SS, Rameshwar P, Siegel A. Effects of hemispheric lateralization and site specificity on immune alterations induced by kindled temporal lobe seizures. Brain Behav Immun 2002;16:706–19.
[9] Neveu PJ, Merlot E. Cytokine stress responses depend on lateralization in mice. Stress 2003;6:5–9.
[10] Raber J, Sorg O, Horn TFW, Yu N, Koob GF, Campbell IL, et al. Inflammatory cytokines: putative regulators of neuronal and neuro-endocrine functions. Brain Res Rev 1998;26:320–6.
[11] Dinarello AD. Biologic basis for interleukin-1 in disease. Blood 1996;87:2095–147.
[12] Peltola J, Hurme M, Miettinen A, Keränen T. Elevated levels of interleukin-6 may occur in cerebrospinal fluid from patients with recent epileptic seizures. Epilepsy Res 1998;31:129–33.
[13] Morganti-Kossmann MC, Satgunaseelan L, Bye N, Kossman T. Modulation of immune response by head injury. Injury 2007;38:1392–400.
[14] Pitkänen A, Sutula TP. Is epilepsy a progressive disorder? Prospects for therapeutic approachers in temporal lobe epilepsy. Lancet Neurol 2002;1:173–81.
[15] Seidenberg M, Kelly KG, Parrish J, Geary E, Dow C, Rutecki P, et al. Ipsilateral and contralateral MRI volymetric abnormatilities in chronic unilateral temporal lobe epilepsy and their clinical correlates. Epilepsia 2005;46:420–30.

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The production of IL-6 in acute epileptic seizure: A video-EEG study

Tiina Alapirtti[a,*], Kai Lehtimäki[b], Riina Nieminen[c], Riikka Mäkinen[d], Jani Raitanen[e,f], Eeva Moilanen[c], Jussi Mäkinen[a], Jukka Peltola[a,g]

[a] Department of Neurology, Tampere University Hospital, Tampere, Finland
[b] Department of Neurosurgery, Tampere University Hospital, Tampere, Finland
[c] The Immunopharmacology Research Group, Faculty of Medicine and Life Sciences, Tampere University Hospital, Tampere, Finland
[d] Department of Clinical Neurophysiology, Pirkanmaa Hospital District, Tampere, Finland
[e] Faculty of Social Sciences, Health Sciences, University of Tampere, Finland
[f] UKK Institute for Health Promotion, Tampere, Finland
[g] Department of Neurology, Faculty of Medicine and Life Sciences, University of Tampere, Tampere, Finland

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ABSTRACT

Experimental and clinical reports highlight the role of cytokines in pathophysiological processes in underpinning epilepsy, but the clinical data remains somewhat limited. The levels of Interleukin (IL)-6 were measured in serum from 49 patients with refractory epilepsy [temporal lobe epilepsy (TLE, n = 23), extratemporal lobe epilepsy (XLE, n = 22), and idiopathic generalized epilepsy (IGE, n = 4)] before and after the first verified seizure (IS; index seizure) during inpatient video-electroencephalographic (VEEG) monitoring. The levels of IL-6 increased significantly at all time points between 3 h and 24 h after the IS compared to the baseline. IL-6 concentrations were significantly higher at the 3 h and 6 h time point after tonic-clonic seizures (TCS) compared to the situation with simple partial and complex partial seizures. An IS duration longer than 100 s, low baseline IL-6 level and < 10 seizures/month in patients with TLE were associated with an increase in IL-6 concentrations during the 24 h after the IS. In patients with TLE, the maximum change in IL-6 levels after IS was significantly higher than in XLE. If the baseline level of IL-6 was low (under 5 pg/ml), seizures induced a significant elevation in both absolute and relative values in TLE patients but not in XLE. In patients with ≤ 10 seizures per month during the last year, the maximum change was higher than in patients with > 10 seizures. If the total seizure burden during registration was ≥ 100 s, the IL-6 increase was significantly higher than if it were under 100 s. The results of this study highlight the complexity of factors involved in the seizure induced production of the inflammatory cytokine, IL-6. The major factor is the epilepsy type i.e. increased production of IL-6 in TLE compared to XLE. The response to a single seizure in TLE is dependent on the previous seizure frequency and the baseline IL-6 concentration.

1. Introduction

Previous studies in patients with epilepsy have addressed both chronic and seizure-induced production of cytokines, especially interleukin-6 (IL-6). In an emergency room (ER) setting mainly in patients with acute symptomatic seizures, IL-6 levels, but not those of IL-1beta (IL-1β) or IL-1 receptor antagonist (IL-1RA), were increased in cerebrospinal fluid (CSF) in patients with tonic-clonic seizures. The plasma concentrations were lower but still reflected the CSF-levels (Peltola et al., 1998; Peltola et al., 2000). In a subsequent ER study IL-6 levels were strongly elevated after recurrent tonic-clonic seizures, whereas after single tonic-clonic or prolonged focal seizures (simple or complex), the levels were increased to a lesser extent (Lehtimäki et al., 2004).

The effects of single seizures in patients with chronic refractory epilepsy have been investigated in a few studies. The levels of IL-1β, IL-1RA, IL-6 and soluble IL-6 receptors (sIL-6R and Gp130) were investigated in plasma after single seizures during video-EEG (VEEG) recordings in 11 patients with chronic localization-related epilepsy (Lehtimäki et al., 2007). The levels of IL-1RA and IL-6 were increased after seizures, whereas those of IL-1β and IL-6 cytokine receptors remained unchanged. That pilot study demonstrated that single seizures can evoke a significant change in IL-6 and IL-1RA plasma levels, but because of the limited sample size, the importance of the epilepsy or seizure type could not be addressed. A subsequent VEEG study with 20 patients further demonstrated that only the temporal lobe epilepsy

* Corresponding author at: Tampere University Hospital, Department of Neurology, PL 2000, 33521 Tampere, Finland.
E-mail address: tiina.alapirtti@pshp.fi (T. Alapirtti).

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(TLE) group displayed a significant increase in plasma levels of IL-6, with concentrations peaking at 6 h postictally (Alapirtti et al., 2009). Postictal levels of IL-1RA and IL-1β did not significantly differ from baseline levels in either of the patients groups.

In a cross-sectional study investigating 86 patients with refractory focal epilepsy, IL-6 concentrations were chronically increased in serum samples from patients (by 11%) compared with healthy controls (0%) ($p = 0.007$) (Liimatainen et al., 2009). Elevated levels of IL-6 were more prevalent in patients with TLE compared to patients with extratemporal lobe epilepsies (XLE) ($p = 0.028$). Furthermore, the mean and the medium levels of IL-6 were higher in patients with TLE than XLE ($p = 0.042$). In contrast, there were no significant differences in the concentrations of IL-1RA between patients and controls.

A recent meta-analysis also demonstrated elevated serum IL-6 levels in TLE patients (de Vries et al., 2016), suggesting that the epilepsy type is important in determining whether or not there is overproduction of cytokines in refractory focal epilepsy. In addition, IL-6 has been postulated to exert neuromodulatory properties which lead to alterations in neuronal network excitability (Vezzani and Viviani, 2015). IL-6 may be one of the general markers of local and systemic immune system activation in specific subtypes of patients with epilepsy, and therefore it is important to clarify in more detail the diverse factors that influence IL-6 responses.

These previous studies had revealed that IL-6 is the most consistently affected cytokine in patients with epilepsy. Multiple factors influence IL-6 production in epilepsy: seizure type and duration, epilepsy type (especially TLE vs XLE) and seizure frequency. However, the relative significance of these factors has remained unknown. In the present study, we have investigated a sufficiently large patient population in order to address this important question.

2. Materials and methods

2.1. The classification of patients

Forty-nine consecutive patients with refractory focal epilepsy admitted to the video-EEG monitoring unit of the Tampere University Hospital were included in the study. The study protocol was approved by the Ethics Committee of the Tampere University Hospital and all the patients signed a written informed consent.

All the patients underwent continuous VEEG monitoring lasting for four days in the electro-clinical characterization of their seizures as part of the routine clinical evaluation before possible epilepsy surgery. Ictal scalp recordings were obtained using synchronous digital video and 24 channel standard bipolar EEG. The electrodes were placed according to the International 10–20 System with additional mastoid and anterior cheek electrodes. All patients underwent a diagnostic brain MRI examination on either a 1.5 (General Electric, Sigma HD, Milwaukee, Wisconsin, USA) or 3.0 (Siemens Healthcare, Magnetom Trio A Tim system 3T, Erlangen, Germany) Tesla machine. Seizures and epileptic syndromes were classified according to the ILAE diagnostic criteria (Commission on Classification and Terminology of the International League Against Epilepsy, 1981). Anticonvulsant medication was discontinued in a stepwise fashion during the monitoring under the supervision of the staff epileptologist.

Localization of the seizure focus was recorded and seizures were categorized as simple partial (SPS), complex partial (CPS) and tonic-clonic seizures (TCS) (including both secondarily and primary generalized tonic-clonic seizures). The first unequivocally verified seizure during the VEEG monitoring was considered as the index seizure (IS). All other seizures after the index seizure during the next 24 h were also registered. Based on the findings in the VEEG recordings and MRI, 23 patients were diagnosed as having TLE, 22 patients had XLE (20 frontal lobe and two parietal lobe epilepsy) and four patients were found to be suffering from idiopathic generalized epilepsy (IGE). Seven patients were on monotherapy, 41 on polytherapy (the mean number of medications was 2.24), and one XLE patient was not being administered any medication. In addition to antiepileptic drugs (AEDs), three patients were being treated with vagus nerve stimulation. One female patient with right hippocampal sclerosis was in the monitoring twice over an interval of 23 months. Clinical data of these three groups of patients are shown in Table 1. The seizure burden was calculated as the total duration of seizures recorded during the 24 h sampling period.

Table 1
Clinical characteristics of patients in video-EEG.

	Temporal lobe epilepsy	Extratemporal lobe epilepsy	Generalized epilepsy
No. of patients	23	22	4
Male/female	12/11	11/11	0/4
Age, mean (range)	40.0 (258)	30.0 (16–52)	32 (27–45)
BMI, mean (± S.D.)	25.9 ± 3.96	25.0 ± 4.59	26.2 ± 3.51
Mean duration of epilepsy, years (range)	23.2 (2–56)	19.6 (1–52)	18.3 (6–32)
Mean seizure frequency/month[a] (range)	8.5 (0.5–30)	59.5 (0.5–240)	1.9 (9.5–4)
Mean number of AEDs	2.17	2.50	2.25
Patient on mono-/polytherapy	4/19	2/19	1/3
MRI findings			
Normal	4	11	3
HS	13	0	Not applicable
Cortical dysplasia	3	5	Not applicable
Other	3	6	1 (vascular lesion)
Index seizure type			
SPS	1	3 (1 SPSE)	Not applicable
CPS	19	15	Not applicable
TCS	3	3	4
Lateralization			
Right	4	11	Not applicable
Left	18	5	Not applicable
Right and left or unknown	1	6	Not applicable
VNS/earlier epilepsy surgery	2/0	1/1	−/−

AED, Antiepileptic drug; CPS, Complex partial seizure; BMI, Body mass index = weight kg/height m^2; HS, Hippocampal sclerosis; TCS, tonic-clonic seizure; SPS, Simple partial seizure; SPSE, Simple partial status epilepticus; VNS, Vagus nerve stimulator.

[a] During the last year.

2.2. Seizures

The mean number of seizures (including the IS) was 2.6 (range: 1–9) in the TLE group and 1.25 (range: 1–2) in the IGE group. In the XLE group, one patient had 180 and another 420 seizures during the 24 h; excluding these patients, the mean number in this group was 5.4 (range: 1–18). Seven patients had only brief seizures during the sampling period. The mean (seconds ± S.D.) duration of the IS in TLE was 405 ± 1491 (median 77), in XLE 58.6 ± 76 (median 26) and in IGE 66 ± 4.0 (median 66). The mean seizure burden (seconds ± S.D.) in the TLE group was 549 ± 1575, in the XLE group it was 1585 ± 5661 and in the IGE group, the mean burden was 81 ± 31.

2.3. Plasma sampling and biochemical analyses

Plasma samples were collected at the onset of video-EEG recording and daily in the morning until patients had their IS and then at 3, 6, 12 and 24 h after the IS. The last sample before the seizure was considered as the baseline sample. The samples were stored at − 70 °C until analyzed. Plasma concentrations of IL-6 were determined by enzyme immunoassay (EIA) with commercial reagents (PeliPair ELISA, Sanquin, Amsterdam, The Netherlands).

Table 2
IL6 level at baseline, and repeated measurements at 3, 6, 12, and 24 h after index seizure.

	n	Baseline IL6 (pg/l)					Measurements 0–24	
		Median	Mean	SD	GMRadj	P_{LTLR}	P_{ANOVA}	$P_{Quadratic}$
Epileptic patients	49	4.22	5.87	7.05			**0.025**^{W}	0.12
Age (mean 40, range 20–58)							0.070^{B}	
≤ 32^{Ref}	25	4.22	6.94	8.94	1.00		0.12^{W}	0.15
> 32	24	4.27	4.76	4.22	0.79	0.42	0.077^{W}	0.55
Syndrome							0.52^{B}	
XLE (male/female 11/13)^{Ref}	22	4.80	5.60	4.08	1.00		0.14^{W}	0.84
TLE (male/female 12/11)	23	4.33	6.76	9.43	0.98	0.95	0.14^{W}	0.30
IGE (male/female 0/4)	4	2.41	2.22	1.46	0.47	0.20	0.22^{W}	0.20
Hippocampal sclerosis (HS)							0.99^{B}	
TLE without HS^{Ref}	9	4.56	9.69	14.2	1.00		0.67^{W}	0.98
TLE with HS	14	4.27	4.88	4.13	0.70	0.51	0.054^{W}	0.19
Etiology							0.81^{B}	
Cryptogenic^{Ref}	18	4.80	5.56	4.01	1.00		0.24^{W}	0.30
Hippocampal sclerosis	14	4.27	4.88	4.13	0.81	0.57	0.054^{W}	0.19
Cortical dysplasia	7	4.22	6.82	6.27	1.20	0.69	0.57^{W}	0.56
Other	10	2.20	7.16	13.4	0.65	0.31	0.27^{W}	0.47
Index seizure type							0.27^{B}	
TCS^{Ref}	10	2.80	2.85	2.23	1.00		0.052^{W}	0.096
CPS, SPS, or SPSE	39	4.44	6.65	7.66	2.30	**0.022**	0.058^{W}	0.52
Lateralization							0.81^{B}	
Right^{Ref}	14	4.39	4.73	2.95	1.00		0.11^{W}	0.40
Left	21	4.21	6.92	9.84	0.90	0.77	0.33^{W}	0.35
Right and left or unknown	14	3.43	5.44	4.77	0.80	0.59	0.21^{W}	0.15
Using enzyme-including AED							0.29^{B}	
No^{Ref}	15	3.33	6.12	5.82	1.00		0.27^{W}	0.17
Weak (oxcarbazepine)	13	4.56	8.09	11.5	1.19	0.66	0.21^{W}	0.15
Strong (carbamazepine/phenytoin)	21	3.53	4.32	3.30	0.87	0.73	0.057^{W}	0.23
Duration of index seizure (median 1 min, range 5 s–5 min)							0.98^{B}	
< 100 s^{Ref}	37	4.22	6.40	7.86	1.00		0.53^{W}	0.65
≥ 100 s	12	3.64	4.25	3.33	0.83	0.60	**0.019**^{W}	0.070
Low baseline IL6 (< 5 pg/ml)							0.60^{B}	
XLE^{Ref}	12	2.21	2.52	1.40	1.00		0.28^{W}	0.76
TLE	15	1.98	2.50	1.55	0.96	0.90	**0.007**^{W}	**0.039**
High baseline IL6 (≥ 5 pg/ml)							0.79^{B}	
XLE^{Ref}	10	8.91	9.30	2.94	1.00		0.35^{W}	0.74
TLE	8	10.1	14.8	12.8	1.58	0.16	0.33^{W}	0.12
≤ 10 seizures/month during last year							0.91^{B}	
XLE^{Ref}	5	4.84	4.62	3.54	1.00		0.28^{W}	0.26
TLE	17	3.18	6.16	10.5	0.93	0.92	**0.020**^{W}	0.083
> 10 seizures/month during last year							0.70^{B}	
XLE^{Ref}	17	4.75	5.89	4.28	1.00		0.50^{W}	0.85
TLE	6	7.35	8.47	5.95	1.81	0.21	0.51^{W}	0.34

Adj GMR: Geometric mean ratio [geometric mean in a group divided by mean in the reference (Ref.) group] adjusted for age and sex.
LTLR: log-transformed linear regression adjusted for age and sex.
ANOVA: Log-transformed repeated measures analysis of variance (B: Test of between-groups effects, W: Test of within-groups effects); Quadratic: Quadratic trend analysis.
H_0 to max: Difference between baseline IL6 value and maximum value after seizure.
MWT: Mann-Whitney test or Kruskal-Wallis test if three or more groups.
AED: Antiepileptic drug.
SD: Standard deviation.
AED: Antiepileptic drug, CPS: Complex partial seizure, IGE: Idiopathic generalized epilepsy, SPS: Simple partial seizure, SPSE: Simple partial status epilepticus, TCS: tonic-clonic seizure, TLE: temporal lobe epilepsy; XLE: extra-temporal lobe epilepsy.
The significant parameters are highlighted with bold characteristics.

3. Statistical analysis

Descriptive statistics (frequencies or means and standard deviations (SD)) were reported to the summarize patients' characteristics. Medians, means and standard error of the means (SEM) were calculated in the comparison of differences between maximum IL-6 value after IS and the baseline concentration of IL-6. Due to the positive skewed distribution, Mann-Whitney *U* test or Kruskal-Wallis test was applied to evaluate the difference between the maximum and baseline IL-6 concentration.

Linear regression analysis was used to assess the difference between age (≤ 32, > 32), epilepsy syndrome, TLE with and without HS, etiology, index seizure type, lateralization, AEDs, and duration of index seizure (< 100, ≥ 100) on log-transformed baseline IL-6 levels. Each model was adjusted for age and sex. Geometric mean ratios from linear regression models were achieved by taking the exponential value of the coefficients. Analysis of variance (ANOVA) for repeated measures was used to investigate between- and within-groups changes in log-transformed IL-6 level over five time points (from baseline to 3, 6, 12, and 24 h).

All analyses were conducted using Stata statistical software version 13.1 (StataCorp, College Station, Texas, USA) and IBM SPSS Statistics for Windows, version 22. In all of the statistical tests, p-values < 0.05 were considered significant.

4. Results

4.1. The baseline levels of IL-6

The baseline levels of IL-6 were not significantly different in the

Table 3
Difference between maximum and baseline concentration of IL-6.

	n	Median	Mean	Std. error	P[a]
Patients with epilepsy	49	0.94	1.81	0.53	
Age					0.38
≤ 32	25	1.21	2.49	0.71	
> 32	24	0.87	1.11	0.79	
Epilepsy syndrome					**0.035**
XLE	22	0.69	0.93	0.20	
TLE	23	1.47	2.19	0.97	
Lateralization of seizure					0.41
Left	21	1.14	1.85	1.04	
Right	14	0.71	1.57	0.44	
IS type					0.32
TCS	10	1.74	3.48	1.49	
SPS/CPS	39	0.93	1.38	0.54	
Duration of epilepsy					0.93
≤ 18	24	0.86	2.71	0.81	
> 18	24	1.10	0.91	0.70	
Seizures per month last year					**0.020**
≤ 10	26	1.52	3.02	0.73	
> 10	23	0.61	0.44	0.69	
Duration of index seizure (sec)					0.16
≤ 65	25	0.81	1.02	0.83	
> 65	24	1.52	2.63	0.63	
Seizure burden, all seizures (including IS, sec)					**0.030**
0–99	17	0.60	0.11	0.91	
100 +	32	1.33	2.71	0.61	
BMI (kg/m^2)					0.78
< 25	22	1.11	1.37	0.26	
≥ 25	27	0.93	2.17	0.95	

BMI = body mass index, CPS = Complex partial seizure, IS = index seizure; TLE = temporal lobe epilepsy; SPS = Simple partial seizure, XLE = extra-temporal lobe epilepsy.
The significant parameters are highlighted with bold characters.
[a] Mann-Whitney test.

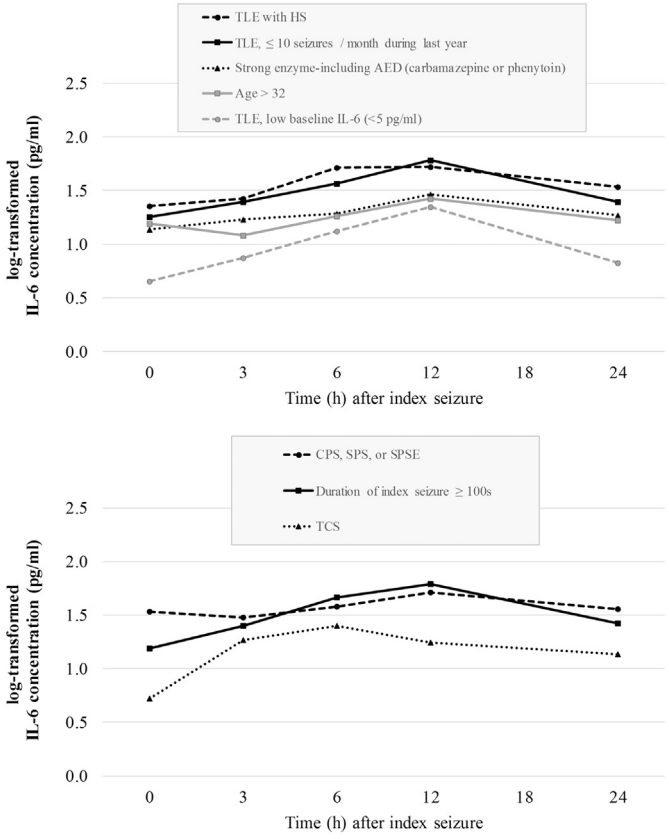

Fig. 1. IL-6 levels before (0 h) and 3, 6, 12, and 24 h after the index seizure A. in patients with TLE with HS; in patients with TLE ≤ 10 seizures/month during last year; in patients using strong enzyme-inducing AED (carbamazepine/phenytoin); in patients with age over 32 years of age; and in patients with TLE, low baseline IL-6 (< 5 pg/ml) B. in patients with SPS, CPS, or SPSE; in patients with duration of the IS ≥ 100 s, and in patients with TCS. AED, Antiepileptic drug; CPS, Complex partial seizure; IS, index seizure; HS, Hippocampal sclerosis; SPS, Simple partial seizure; SPSE, Simple partial status epilepticus, TCS, tonic-clonic seizure; TLE, Temporal lobe epilepsy.

patients with the three distinctive types of epilepsy (Table 2). There was no effect of pre-VEEG seizure frequency on baseline IL-6 concentrations. No associations were detected between the baseline levels IL-6 and the patient's age, etiology, the use of enzyme-including AED, duration of epilepsy, duration of index seizure or lateralization. In patients with focal simple or complex partial seizure as the IS, the IL-6 baseline concentration was significantly higher compared to patients with TCS as their IS (6.65 vs. 2.85 pg/ml, unadjusted model p = 0.024 and sex- and age-adjusted model p = 0.022).

4.2. Seizure induced production of IL-6

4.2.1. The postictal levels of IL-6 in the time sequence with reference to seizure types

The levels of IL-6 were significantly increased at all time points between 3 h and 24 h after the IS compared to the baseline when all seizures were analyzed (p = 0.032 3 h, p = 0.019 6 h, p < 0,001 12 h and 24 h; Wilcoxon signed ranks test).

Levels of IL-6 were significantly higher at the 3 h (p = 0.007) and 6 h (p = 0.053) time points after TCS in comparison to SPS and CPS. Postictal levels of IL-6 were higher at the 3 h time point after focal onset TCS (P = 0.019) and primary generalized TCS (P = 0.040) when compared to CPS. Levels of IL-6 were significantly higher at the 6 h time point after TCS compared to those after SPS at the same time; respective P values 0.028 and 0.050.

4.2.2. Difference between maximum and baseline concentrations of IL-6

There was a significant difference between TLE and XLE with an elevated plasma level of IL-6 in patients with TLE (Table 3). The patients with frequent seizures displayed a smaller increase after single seizures compared with those with infrequent seizures (Fig. 1). The higher the total seizure burden during the registration, the greater the IL-6 response.

No correlation was found between the production of IL-6 and the duration of epilepsy or BMI in any patient or in seizure type subgroups. Lateralization of epilepsy exerted no influence on the absolute change of IL-6 levels, in patients with left lateralization, the relative change was higher than in patients with right lateralization (mean 1.17 vs 0.44; median 0.28 vs 0.17), but the difference was not statistically significant (Mann-Whitney test p = 0.47 and 0.84).

The following characteristics were associated with an increase in IL-6 concentrations during 24 h after the IS (Fig. 1); 1) a duration of the index seizure longer than 100 s, 2) a low baseline IL-6 and 3) fewer than 10 seizures/month in patients with TLE were associated with an increase in IL-6 concentrations during 24 h after the IS (Table 2). There was also an increasing tendency for elevated IL-6 levels between repeated measurements of IL-6 levels after the IS in older patients (p = 0.077), as well as in TLE patients with hippocampal sclerosis (p = 0.054) and in patients using strong enzyme-including AEDs (p = 0.057).

4.2.3. The effect of baseline IL-6 concentration on the seizure induced production in TLE and XLE

If the baseline level of IL-6 was low (under 5 pg/ml), the IS induced a significantly greater elevation in both absolute and relative IL-6 concentrations in patients with TLE but not in those with XLE (median relative change 1.10 vs 0.28, p = 0.006 Independent Samples Median Test; median absolute change 3.01 vs 0.52; Mann-Whitney *U* test

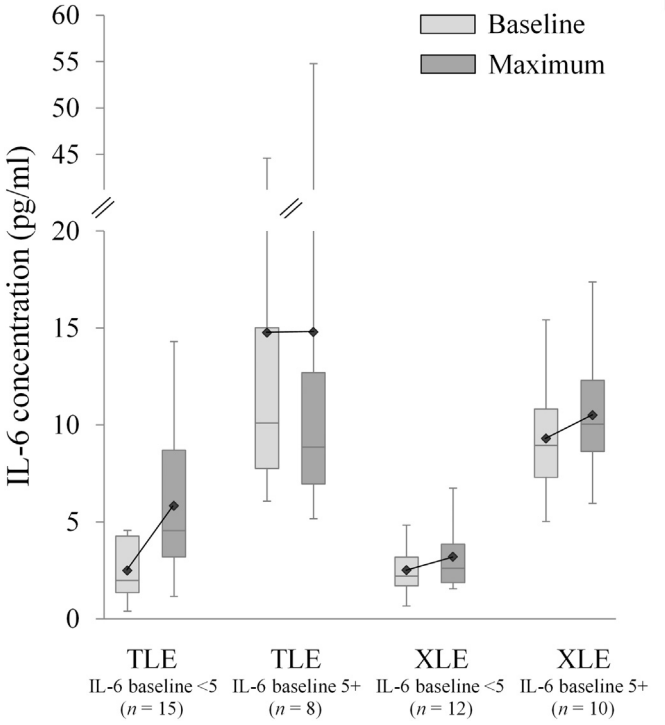

Fig. 2. The effect of baseline IL-6 concentration on the seizure induced maximum production in TLE and XLE. Box and whisker plot legend: midline = median, dot = mean, box = 25th and 75th percentiles, whiskers = min and max values.

p = 0.004) (Fig. 2). In terms of the maximum increase from the baseline IL-6 level (i.e. the highest IL-6 level assayed in the four measurements after IS minus the baseline level), Mann-Whitney U test also confirmed the difference between TLE and XLE patients with a low baseline IL-6 concentration (< 5 pg/ml) (p = 0.003). The trend analysis also showed a significant quadratic trend (downward U-shape) for patients with TLE and low baseline IL-6 levels (Table 2, p = 0.039). There were no similar results if the TLE or the XLE patients exhibited a high baseline IL-6 concentration (median absolute change 0.59 vs 0.86, NS; median relative change 0.07 vs 0.52; NS). In XLE, the baseline level had no influence on IL-6 concentrations after the IS (Table 4).

5. Discussion

This study provides new information about the regulation of IL-6 levels in patients with refractory epilepsy. The most important new finding in the present study is that seizure related serum IL-6 production is dependent on the baseline IL-6 levels in patients with TLE. On the other hand, high baseline levels are related to the seizure frequency.

Patients who had experienced fewer than 10 monthly seizures during the previous year showed a significant increase in IL-6 level after the IS. In a subgroup analysis, it was found that patients with TLE and infrequent seizures exhibited a significant increase in IL-6 level after the IS, but there was no similar effect in XLE. There has been reported to be some patients with TLE who have chronically elevated IL-6 concentrations (Liimatainen et al., 2009). In our study the patients with low levels of IL-6 responded to single seizures with a significant IL-6 raise. These findings seem to be specific to TLE, since patients with XLE do not show similar findings. Patients experiencing longer seizures lasting at least 100 s, had elevated IL-6 levels postictally compared with the patients with shorter seizures. We also confirmed a previous finding that seizures elevate IL-6 concentration postictally. We also noted that seizure type exerted an effect on baseline IL-6 levels, i.e. IL-6 levels were higher in patients with TCS. Finally it was observed that the maximum change in the IL-6 level after IS was significantly greater in TLE than in XLE.

The present study provides additional data to highlight the differences between TLE and XLE. In a previous study only in the TLE group was an increase in plasma levels of IL-6 observed (Alapirtti et al., 2009). Here, in a substantially larger number of patients, the maximum elevation in the serum IL-6 levels after IS was significantly greater in TLE than in XLE. In another study, the serum levels of IL-6 were chronically elevated in epilepsy patients compared to healthy controls, and serum levels of IL-6 were higher in patients with TLE compared to XLE (Liimatainen et al., 2009). In that study, there was a subgroup of patients with TLE, who had IL-6 levels over 5 pg/ml, but all of the patients with XLE or healthy controls had levels below 5 pg/ml. In our study in patients with a low baseline IL-6 level (under 5 pg/ml), seizures induced a higher maximum elevation in both absolute and relative values of IL-6 in patients with TLE but not in those with XLE. This was not the case if TLE or XLE patient had a high baseline IL-6 concentration. As far as we are aware, this is the first study to demonstrate that lower chronic IL-6 levels in TLE are associated with a more profound IL-6 response after a single seizure. In contrast, if the baseline IL-6 level was already high, there was no major IL-6 response after the seizure.

TLE is the most common focal epilepsy type, and the temporal lobe is vulnerable in many conditions affecting the brain. There is ample evidence to suggest that TLE may be a progressive disease and lead to hippocampal atrophy (Pitkänen and Sutula, 2002). Peripheral IL-6 activation may reflect this insidious pathology. If in the TLE IL-6 concentration is constantly elevated, the levels of this cytokine are not elevated by a seizure, which may be attributable to the ongoing active inflammation process. In a previous study in VEEG settings, all patients with elevated CRP had TLE vs. none in XLE (Alapirtti et al., 2012). There is another study reporting elevated concentrations of two markers of brain damage, neuron-specific enolase and S-100b protein postictally in TLE but not in XLE patients, supporting also the hypothesis that seizures may cause neuronal damage in TLE and this damage is reflected in peripheral blood and CSF measurements of these biomarkers (Palmio et al., 2008). According to experimental studies, IL-6 is considered as a proconvulsant compound (Kalueff et al., 2004). In TLE, there may be subgroups of patients, with some having more intense chronic inflammation with higher levels of IL-6 without any observable incremental effects after a single seizure. This immunological response in TLE also correlates with the seizure frequency noted here; in patients with < 10 seizures per month during the last year, the maximum change after a single seizure was higher than in patients with more frequent seizures.

The results from the present study also confirm previous findings on

Table 4
The effect of baseline IL-6 concentration on the seizure induced production in TLE and XLE.

	TLE			XLE		
	N	Mean (sem)	Med (min/max)	N	Mean (sem)	Med (min/max)
Mean relative change in patients with low baseline IL-6 (< 5)	15	2.06 (0.61)	1.10 (− 0.01/8.62)	12	0.39 (0.15)	0.28 (− 0.28/1.58)
Mean relative change in patients with high baseline IL-6 (5 +)	8	− 0.01 (0.10)	0.07 (− 0.73/0.23)	10	0.14 (0.05)	0.10 (0.03/0.60)
Mean absolute change in patients with low baseline IL-6 (< 5)	15	3.34 (0.76)	3.01 (− 0.01/9.74)	12	0.68 (0.19)	0.52 (− 0.59/1.90)
Mean absolute change in patients with high baseline IL-6 (5 +)	8	0.04 (2.30)	0.59 (− 13.7/10.2)	10	1.23 (0.37)	0.86 (0.22/4.19)

IL-6 reactions after epileptic seizures. These published studies with smaller patient groups have revealed increased plasma IL-6 levels more often after TCS than after complex partial seizures in video-EEG (Lehtimäki et al., 2007). The present study with a larger patient population detected increased levels of IL-6 at all time points between 3 h and 24 h after the IS compared to the baseline. The increase of IL-6 levels was more prominent after the more severe seizure types at the 3 h and 6 h time points In TCS, the seizure activity spreads throughout the brain, whereas in SPS and CPS, the epileptic activity is restricted to the seizure focus and associated structures, and there may be less leakage through the BBB to plasma. Unlike in previous reports (Alapirtti et al., 2009; Lehtimäki et al., 2007), in the present study, postictally increased IL-6 levels had still not returned to the baseline level within 24 h. Postictal IL-6 levels remained elevated also in another video-EEG study (Bauer et al., 2009). After TCSs Uludag et al. (2013) reported higher plasma IL-6 levels at 24 h than at baseline (Uludag et al., 2013). Bauer et al. (Bauer et al., 2009) measured higher levels after right-sided seizures as compared to left-sided, but in our study there was no difference between the lateralization of the seizure focus. A previous study reported that the C-reactive protein (CRP) concentration was significantly higher in patients with refractory focal epilepsy than in controls, and after a single seizure, the most important predictor of an increase in CRP level was that the seizure was tonic-clonic type (Alapirtti et al., 2012). Analysis of CSF would have been interesting in our present evaluation, but unfortunately, it was not possible to collect these samples during video-EEG monitoring.

The strength of our study is that video-EEG provides opportunity of exactly defining in detail the extent, localization and duration of seizures in a controlled environment. By examining patients with chronic epilepsy, it was also possible to exclude acute causes of seizures, e.g. trauma, infections, stroke, which may themselves also induce cytokine production. On the other hand as a limitation to our study we have only a few patients in the idiopathic generalized epilepsy group making it more difficult to draw definitive conclusions concerning that epilepsy type. The results of this study highlight the complexity of factors involved in the seizure induced production of the inflammatory cytokine, IL-6. The major factor is the epilepsy type of the focal epilepsies, with increased production of IL-6 in TLE compared to XLE. The response to a single seizure in TLE is dependent on the previous seizure frequency and IL-6 concentration. There is no elevation in IL-6 levels after a single seizure in patients with frequent seizures, instead these patients display a chronically elevated IL-6 concentration. In contrast, patients with infrequent seizures and who have low baseline IL-6 levels exhibit a significant elevation in this cytokine's level after a single seizure. All patients suffering TCS (both focal and generalized) show an augmented response in comparison to patients with other seizure types.

Disclosures

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References

Alapirtti, T., Rinta, S., Hulkkonen, J., Mäkinen, R., Keränen, T., Peltola, J., 2009. Interleukin-6, interleukin-1 receptor antagonist and interleukin-1beta production in patients with focal epilepsy: a video-EEG study. J. Neurol. Sci. 280, 94–97.

Alapirtti, T., Waris, M., Fallah, M., Soilu-Hänninen, M., Mäkinen, R., Kharazmi, E., Peltola, J., 2012. C-reactive protein and seizures in focal epilepsy: a video-electroencephalographic study. Epilepsia 53, 790–796.

Bauer, S., Cepok, S., Todorova-Rudolph, A., Nowak, M., Koller, M., Lorenz, R., Oertel, W.H., Rosenow, F., Hemmer, B., Hamer, H.M., 2009. Etiology and site of temporal lobe epilepsy influence postictal cytokine release. Epilepsy Res. 86, 82–88.

Proposal for revised clinical and electroencephalographic classification of epileptic seizures. From the Commission on Classification and Terminology of the International League Against Epilepsy. Epilepsia 22, 489–501.

Kalueff, A.V., Lehtimäki, K.A., Ylinen, A., Honkaniemi, J., Peltola, J., 2004. Intranasal administration of human IL-6 increases the severity of chemically induced seizures in rats. Neurosci. Lett. 365, 106–110.

Lehtimäki, K.A., Keränen, T., Huhtala, H., Hurme, M., Ollikainen, J., Honkaniemi, J., Palmio, J., Peltola, J., 2004. Regulation of IL-6 system in cerebrospinal fluid and serum compartments by seizures: the effect of seizure type and duration. J. Neuroimmunol. 152, 121–125.

Lehtimäki, K.A., Keränen, T., Palmio, J., Mäkinen, R., Hurme, M., Honkaniemi, J., Peltola, J., 2007. Increased plasma levels of cytokines after seizures in localization-related epilepsy. Acta Neurol. Scand. 116, 226–230.

Liimatainen, S., Fallah, M., Kharazmi, E., Peltola, M., Peltola, J., 2009. Interleukin-6 levels are increased in temporal lobe epilepsy but not in extra-temporal lobe epilepsy. J. Neurol. 256, 796–802.

Palmio, J., Keränen, T., Alapirtti, T., Hulkkonen, J., Makinen, R., Holm, P., Suhonen, J., Peltola, J., 2008. Elevated serum neuron-specific enolase in patients with temporal lobe epilepsy: a video-EEG study. Epilepsy Res. 81, 155–160.

Peltola, J., Hurme, M., Miettinen, A., Keränen, T., 1998. Elevated levels of interleukin-6 may occur in cerebrospinal fluid from patients with recent epileptic seizures. Epilepsy Res. 31, 129–133.

Peltola, J., Palmio, J., Korhonen, L., Suhonen, J., Miettinen, A., Hurme, M., Lindholm, D., Keränen, T., 2000. Interleukin-6 and interleukin-1 receptor antagonist in cerebrospinal fluid from patients with recent tonic-clonic seizures. Epilepsy Res. 41, 205–211.

Pitkänen, A., Sutula, T.P., 2002. Is epilepsy a progressive disorder? Prospects for new therapeutic approaches in temporal-lobe epilepsy. Lancet Neurol. 1, 173–181.

Uludag, I.F., Bilgin, S., Zorlu, Y., Tuna, G., Kirkali, G., 2013. Interleukin-6, interleukin-1 beta and interleukin-1 receptor antagonist levels in epileptic seizures. Seizure 22, 457–461.

Vezzani, A., Viviani, B., 2015. Neuromodulatory properties of inflammatory cytokines and their impact on neuronal excitability. Neuropharmacology 96, 70–82.

de Vries, E.E., van den Munckhof, B., Braun, K.P., van Royen-Kerkhof, A., de Jager, W., Jansen, F.E., 2016. Inflammatory mediators in human epilepsy: a systematic review and meta-analysis. Neurosci. Biobehav. Rev. 63, 177–190.

Epilepsia, **(*):1–7, 2012
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FULL-LENGTH ORIGINAL RESEARCH

C-reactive protein and seizures in focal epilepsy: A video-electroencephalographic study

*Tiina Alapirtti, †Matti Waris, *Mahdi Fallah, ‡Merja Soilu-Hänninen, §Riikka Mäkinen, *Elham Kharazmi, and *Jukka Peltola

*Department of Neuroscience and Rehabilitation, Tampere University Hospital, Tampere, Finland; Departments of †Virology and ‡Neurology, University of Turku, Turku, Finland; and §Department of Clinical Neurophysiology, Pirkanmaa Hospital District, Tampere, Finland

Summary

Purpose: C-reactive protein (CRP) has been studied extensively in many noninflammatory neurologic conditions, but there has been little study of CRP in the context of seizures or epilepsy. The purpose of this study was to examine CRP concentrations in patients with refractory focal epilepsy who were undergoing video-electroencephalography (EEG) monitoring compared with healthy controls, and CRP change during 24 h after a seizure.

Methods: CRP levels were measured in serum at the onset of video-EEG recording (CRP-0h) and at 3, 6, 12, and 24 h after index seizure (the first verified localized-onset seizure) in 31 patients during inpatient video-EEG monitoring by using high sensitivity measurement of CRP concentration. The patients were categorized into two groups: temporal lobe epilepsy (TLE; n = 15) and extratemporal lobe epilepsy (XLE; n = 16). Eighty healthy volunteers served as controls.

Key Findings: CRP-0h concentration was significantly higher in patients with refractory focal epilepsy than in controls (3.5 vs. 0.7 mg/ml, p < 0.001). All five patients with elevated CRP-0h (>mean + 2 standard deviations in controls) had TLE (vs. none in XLE; p = 0.018). Index seizure type was associated with CRP increase from baseline to maximum level after index seizure (p = 0.005). The most important predictor of increase in CRP level was secondarily generalized tonic–clonic seizure (SGTCS; p = 0.030).

Significance: The higher baseline levels in patients with epilepsy compared with healthy controls demonstrates that CRP concentrations are also affected in refractory epilepsy. Our data suggest that SGTCS stimulates CRP production. These results emphasize the association between inflammation and refractory epilepsy.

KEY WORDS: Refractory epilepsy, Temporal lobe epilepsy, Video-EEG, C-Reactive protein, Seizure, Interleukin-6.

C-reactive protein (CRP) is an acute-phase short pentraxin protein that is produced mainly in the liver in response to inflammatory signals, most prominently interleukin-6 (IL-6) (Mantovani et al., 2008). CRP was recognized initially as an inflammatory marker, but recently it has been studied extensively in many noninflammatory neurologic conditions; however, there has been little study of CRP in the context of seizures or epilepsy.

Elevated CRP level is a well-known biomarker of atherosclerotic cardiovascular disease risk (Ballantyne et al., 2005). In addition, CRP appears to play a role in neurodegenerative diseases. In dementia, higher levels of markers of inflammation such as CRP (Engelhart et al., 2004) and IL-6 have been reported (Dziedzic, 2006). High CRP concentrations are also detected in patients with mild cognitive impairment, which seems to predict the development of dementia years later (Xu et al., 2009). The same phenomenon is also observed in children with Down syndrome and later onset of dementia (Licastro et al., 2005). In experimental models it has been suggested that elevated CRP level even contributes to memory loss and early phase of pathogenesis of Alzheimer's disease (Lin et al., 2009).

There is accumulating evidence of inflammation in epilepsy. Increased IL-6 (Hulkkonen et al., 2004) and CRP (Peltola et al., 2002) concentrations have been measured in patients with previously undiagnosed and untreated tonic–clonic seizures without any evidence of systemic or central nervous system infections. IL-6 concentration was also chronically increased in patients with epilepsy compared with healthy controls, more prevalently in patients with temporal lobe epilepsy (TLE) compared with extratemporal lobe epilepsy (XLE) (Liimatainen et al., 2009). TLE with recurring seizures may be a progressive disorder leading to neuropathologic changes with increasing severity of epilepsy and

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Address correspondence to Tiina Alapirtti, Department of Neurosciences and Rehabilitation, Tampere University Hospital, PO Box 2000, FI-33521 Tampere, Finland. E-mail: tiina.alapirtti@pshp.fi

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cognitive decline (Pitkänen & Sutula, 2002). In our previous study, patients with refractory TLE had increased plasma IL-6 concentrations after seizures, whereas in XLE patients there was no change (Alapirtti et al., 2009).

According to a recent study, antiepileptic drugs (AEDs) influence CRP concentrations depending on their liver enzyme–inducing properties. In patients with epilepsy on a monotherapy regimen, switch from either phenytoin or carbamazepine to noninducing AEDs produces significant declines in CRP (−31.4%; p = 0.027) (Mintzer et al., 2009). The association between CRP level and use of enzyme-inducing drugs in patients with refractory epilepsy, however, remains unknown.

The aims of this study were (1) to ascertain whether CRP levels change after an acute seizure in patients with refractory epilepsy; (2) to evaluate the difference in baseline CRP levels between patients and healthy controls; and (3) to assess whether elevated CRP levels or change in levels is associated with epilepsy syndrome, etiology, epilepsy duration, seizure type, seizure duration, seizure frequency prior to the seizure, or current use of enzyme-inducing AEDs such as phenytoin or carbamazepine.

Methods

Classification of patients

Thirty-one patients with refractory focal epilepsy admitted to the video-electroencephalography (EEG) monitoring unit of the Tampere University Hospital were included in the study. All patients provided written informed consent. The study protocol was approved by the ethics committee of the Tampere University Hospital.

All the patients underwent continuous video-EEG monitoring for 4 days for electroclinical characterization of their seizures as part of the routine clinical evaluation for possible epilepsy surgery. Ictal scalp recordings were obtained using synchronous digital video and 24-channel standard bipolar EEG. Electrodes were placed according to the International 10–20 System with additional mastoid and anterior cheek electrodes. All patients underwent a diagnostic brain magnetic resonance imaging (MRI) examination on a 1.5 Tesla machine (General Electric Signa HD, Milwaukee, WI, U.S.A.). Seizures and epileptic syndromes were classified according to the International League Against Epilepsy (ILAE) diagnostic criteria (Commission on Classification and Terminology of the International League Against Epilepsy, 1981). An anticonvulsant medication was discontinued in a stepwise fashion during the monitoring by evaluation of the staff epileptologist.

Localization of the seizure focus was recorded, and seizures were categorized as simple partial (SPS), complex partial (CPS), or secondarily generalized tonic–clonic seizure (SGTCS). The first unequivocally verified localized onset seizure during the video-EEG monitoring was considered as the index seizure. All other seizures after the index seizure during the next 24 h were also registered. Baseline characteristics of patients are presented in Table 1.

Controls

Eighty healthy volunteers served as controls (66 women and 14 men; mean age 30 years, range 19–55 years). Data on volunteers were presented in more detail in a previous study (Laihia et al., 2005).

Blood sampling and biochemical analyses

Blood samples were collected at the beginning of the 4-day recordings, and at 3, 6, 12, and 24 h after the index seizure. The serum samples were stored at −70°C prior to analysis. High sensitivity measurement of CRP was performed in Department of Virology, University of Turku, Finland, as described earlier (Koskinen et al., 2004). High sensitivity measurement of CRP, also referred as hsCRP, was used to detect lower concentrations of the protein more accurately than the standard test, which measures a much wider range of CRP levels but is less sensitive in the lower range.

Statistical analysis

Because of a positive skewness in the distribution of CRP levels, log-transformation was applied to almost all of the analyses. The p-value and geometric mean ratio from log-transformed linear regression analysis of CRP level (adjusted for age and sex) were calculated using STATA software, version 8 (StataCorp LP, College Station, Texas, U.S.A.). CRP levels were also categorized into normal and

Table 1. Clinical characteristics of patients

Epilepsy syndrome	No of patients	Male/ female	Age, mean (range)	Mean duration of epilepsy, years (range)	Mean seizure frequency/ month* (range)	Patients receiving monotherapy/ polytherapy	MRI findings				Index seizure type		
							Normal	HS	Cortical dysplasia	Other	SPS	CPS	SGTCS
Temporal lobe epilepsy	15	8/7	40.3 (20–58)	24.2 (2–56)	7.4 (0.5–26)	3/12	4	10	1	1	1	11	3
Extratemporal epilepsy	16	7/9	28.3 (16–52)	16.2 (1–52)	40.8 (0.5–200)	3/12	8	–	4	4	4	10	2

HS, hippocampal sclerosis; SPS, simple partial seizure; CPS, complex partial seizure; SGTCS, secondary generalized tonic–clonic seizure.
*During the last year.

elevated by cutoff of mean CRP level plus two standard deviations (SDs) in controls (5.8 mg/l). Logistic regression was used for binomial CRP seropositivity data analysis. When logistic regression analysis was not applicable due to zero cells, Fisher's exact test was performed. Log-transformed repeated-measures analysis of variance (ANOVA) was performed, using MedCalc software version 10.4.0.0 (MedCalc Software, Mariakerke, Belgium), to assess the change in CRP levels from baseline to 3, 6, 12, and 24 h after index seizure. Linear and quadratic trend analyses were performed to identify trend of CRP values after index seizure. Variables such as age, duration of epilepsy, duration of index seizure, and number of AEDs were used as continuous variables in the analyses when applicable. For illustration purposes and analyzing between-groups and within-group effects these variables were categorized into two groups based on their mean or median depending on their distribution.

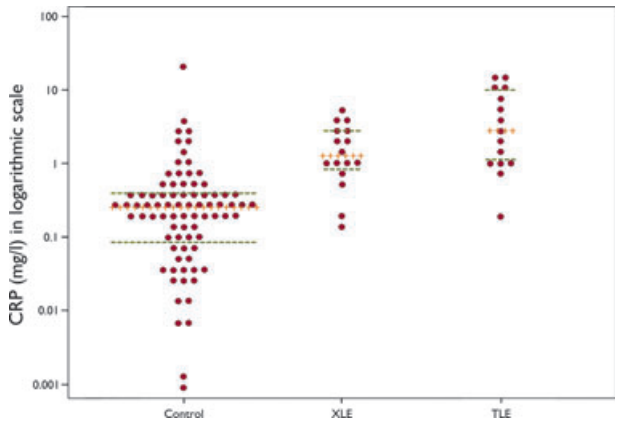

Figure 1.
C-reactive protein level in healthy controls and patients by syndrome (TLE, temporal lobe epilepsy; XLE, extra-TLE; +++ = median, – – – = interquartile range).
Epilepsia © ILAE

Results

Based on the findings in the video-EEG recordings and MRI, 15 of the patients had TLE and 16 patients had XLE (14 frontal lobe and 2 parietal lobe epilepsy). Six patients were receiving monotherapy, 24 were receiving polytherapy (the mean number of medications 2.3), and one patient had no medication. Three patients were treated in addition to AEDs with vagus nerve stimulation. AEDs were classified into three categories based on their enzyme-inducing properties: noninducing, weak, and strong enzyme inducer. One of the patients with XLE was taking oral contraception hormone therapy, one had transdermal hormone replacement therapy, and two had levonorgestrel intrauterine device. In the TLE group, one patient had transdermal hormone replacement therapy and one had levonorgestrel intrauterine device. Three of the patients with TLE were smokers; five of the patients with XLE were smokers. None of the patients were receiving statin therapy. There was no substantial difference in body mass index between the groups (TLE: mean 25.4 ± 3.5 kg/m^2, median 26.0 kg/m^2; XLE: mean 25.3 ± 4.0 kg/m^2, median 25.4 kg/m^2).

Cases versus controls at baseline

The baseline serum level of CRP (CRP-0h) was significantly higher in patients with refractory epilepsy compared with controls (3.5 vs. 0.7 mg/ml, $p < 0.001$). All patients with epilepsy had CRP-0h level higher than 0.1 mg/L (Fig. 1). Mean CRP-0h was higher in the TLE group than in the XLE group, but this difference was not statistically significant (sex- and age-adjusted geometric mean ratio: 1.5, 95% CI 0.6–3.9). When CRP-0h levels were converted to a binomial variable, all five patients with elevated CRP-0h (>5.8 mg/L [mean + 2 SD in controls] had TLE, and only one control had elevated CRP-0h (TLE vs. none in XLE, $p = 0.018$; cases vs. control $p = 0.006$; TLE vs. control, $p < 0.001$; TLE + hippocampal sclerosis [HS] vs. TLE-HS, $p = 0.600$; Fig. 1).

In patients with epilepsy with strong enzyme inducers (carbamazepine or phenytoin) the mean CRP-0h levels were twice as high as in patients with noninducing drugs, but this difference did not reach statistical significance ($p = 0.084$).

Higher CRP-0h levels were associated with lower numbers of AEDs ($p < 0.001$). Higher CRP-0h levels were also detected in patients with older age at diagnosis (or shorter duration; $p = 0.058$) (Table 2). In our sample, baseline CRP level did not show any significant association with sex, etiology, seizure frequency during the year before index video-EEG, index seizure type, or duration of index seizure. Older subjects (patients + controls) had higher CRP-0h levels ($p = 0.021$). There was no statistically significant difference between men and women in terms of body mass index (26.5 and 24.3, respectively; $p = 0.100$).

Seizure-related changes

CRP level also increased significantly during 24 h after the index seizure in four out of five patients with SGTCS as index seizure, particularly after 3 h (ANOVA; $p = 0.030$; Figs. 2 and 3). The overall pattern of CRP change after index seizure was U shaped with an initial decrease in the first 3 h and an increase afterward, mainly after 12 h (Fig. 2). In general, the trend analysis showed a significant quadratic trend when all 31 patients were analyzed together ($p = 0.029$; Table 2). Quadratic trend was significant in some subgroups of patients such as TLE syndrome and duration of index seizure ≥1 min (Table 2).

Because index seizure type had significant interaction in log-transformed repeated measures analyses of variance, the analyses were repeated for each seizure type strata (test of within-groups effects in Table 2). Mann-Whitney (rank-sum) test also confirmed the difference between index

Table 2. C-reactive protein level at baseline and repeated measurements at 3, 6, 12, and 24 h after index seizure

		Baseline (0 h) C-reactive protein (mg/L)					Measurements 0–24		0 h to max
	n	Median	Mean	SD	Adj GMR	P LTLR	P ANOVA	P Quadratic	P MWT
All	111	0.3	1.5	3.3		<0.001			
Healthy controls[Ref]	80	0.3	0.7	2.6	1.0				
Patients with epilepsy	31	2.0	3.5	4.0	10.1		0.224[W]	0.029	
Age (mean 34, range 16–58)					1.04	0.033[a]	0.003[B]		0.451
≤34[Ref]	17	1.2	2.6	2.9			0.522[W]	0.522	
>34	14	2.4	4.7	5.0			0.369[W]	0.369	
Syndrome						0.378	0.060[B]		0.874
XLE[Ref]	16	1.2	2.0	1.7	1.0		0.888[W]	0.518	
TLE	15	2.8	5.2	5.1	1.5		0.222[W]	0.035	
Hippocampal sclerosis (HS)						0.786	0.920[B]		0.953
TLE without HS[Ref]	5	2.8	4.9	5.4	1.0		0.366[W]	0.196	
TLE with HS	10	2.8	5.3	5.3	0.8		0.391[W]	0.125	
Etiology							0.191[B]		0.574
Cryptogenic[Ref]	11	2.0	3.5	4.0	1.0		0.387[W]	0.122	
Hippocampal sclerosis	10	2.8	5.3	5.3	1.2	0.797	0.391[W]	0.125	
Cortical dysplasia	5	1.0	1.2	1.0	0.4	0.112	0.356[W]	0.224	
Other	5	2.0	2.4	1.4	1.6	0.448	0.377[W]	0.394	
Index seizure type						0.317	0.884[B]		0.005
Generalized (SGTCS)[Ref]	5	1.2	2.0	2.1	1.0		0.030[W]	0.073	
Partial (CPS, SPS, or SPSE)	26	2.0	3.8	4.3	1.7		0.157[W]	0.202	
Duration of index seizure (median 1 min, range 5 s to 5 min)		0.8	0.267*		0.160[B]		0.081		
<1 min[Ref]	12	1.6	2.1	1.7			0.033[W]	0.739	
≥1 min	19	2.0	4.4	4.8			0.132[W]	0.029	
Using enzyme-inducing AED							0.458[B]		0.287
No[Ref]	8	1.4	2.4	2.4	1.0		0.820[W]	0.937	
Weak (oxcarbazepine)	10	1.9	2.4	2.1	1.1	0.875	0.179[W]	0.107	
Strong (carbamazepine/phenytoin)	13	2.0	5.1	5.4	1.6	0.385	0.246[W]	0.084	

*The variable was used as continuous in the analysis. Adj GMR, geometric mean ratio [geometric mean in a group divided by mean in the reference (Ref) group] adjusted for age and sex; LTLR, log-transformed linear regression adjusted for age and sex. ANOVA, log-transformed repeated-measures analysis of variance (B: test of between-groups effects; W, test of within-groups effects); quadratic, quadratic trend analysis; 0 h to max, difference between baseline CRP value and maximum value after seizure; MWT: Mann-Whitney test; AED, antiepileptic drug; SD, standard deviation; SGTCS, secondarily generalized tonic–clonic seizure; SPS, simple partial seizure, CPS, complex partial seizure, and SPSE, simple partial status epilepticus.

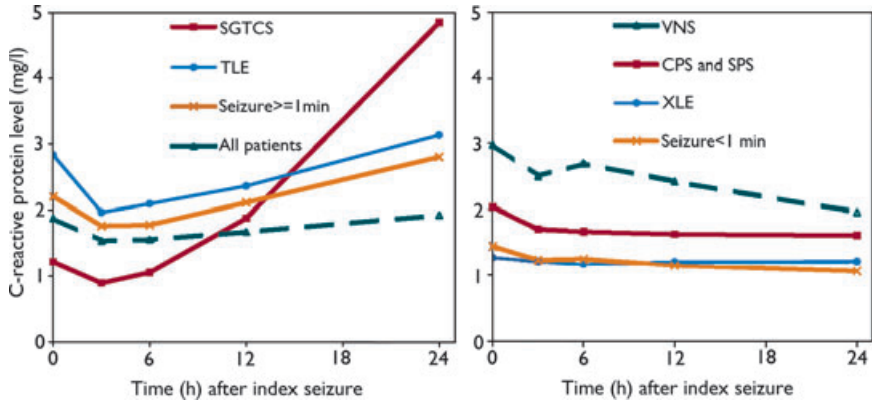

Figure 2.
Geometric mean trend of C-reactive protein level at baseline (0h) and 3, 6, 12, and 24 h after index seizure (SGTCS, secondarily generalized tonic–clonic seizure, TLE, temporal lobe epilepsy; XLE, extra-TLE; VNS, vagus nerve stimulation). *Epilepsia* © ILAE

seizure type (p = 0.005) in terms of maximum difference with baseline CRP level (maximum CRP level among four measurements after index seizure minus baseline level).

In line with baseline CRP level, older patients (p = 0.003) had a significantly higher mean of all five CRP measurements during the baseline and 24 h measurements after the index seizure (in logarithmic scale; test of between-groups effects in Table 2). In patients with vagus nerve stimulation, CRP was not higher than in other patients with epilepsy.

Discussion

The major findings in our study are related to seizure-induced differences in CRP levels as well as to the baseline

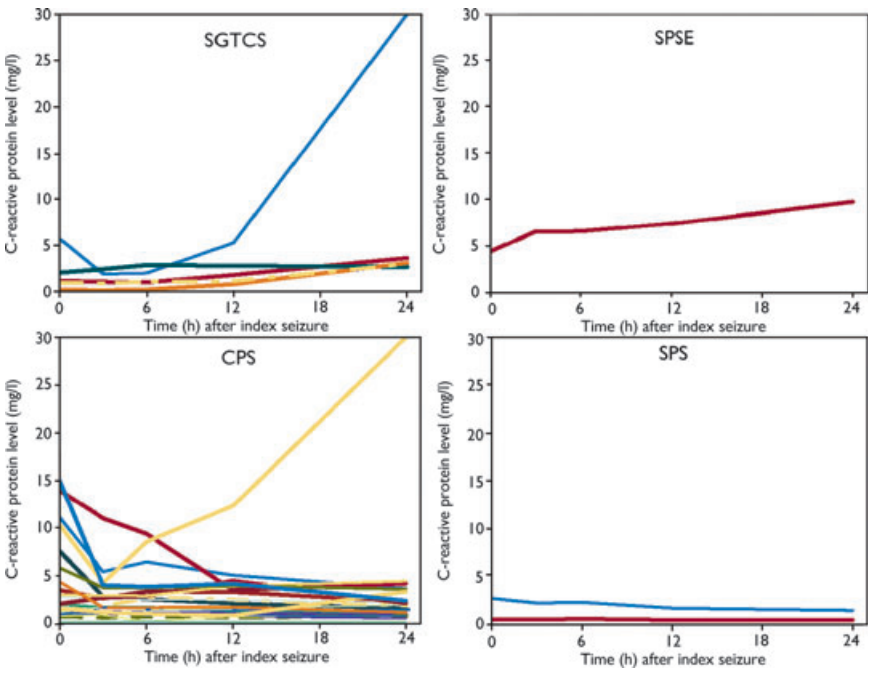

Figure 3.
Individual change in C-reactive protein level at baseline (0 h), and 3, 6, 12, and 24 h after index seizure type (SGTCS, secondarily generalized tonic–clonic seizure; SPS, simple partial seizure; CPS, complex partial seizure; SPSE, simple partial status epilepticus). *Epilepsia* © ILAE

differences. SGTCS caused a significant increase in CRP levels, whereas SPS or CPS did not. Moreover, the baseline levels in patients with refractory epilepsy are sixfold higher compared with healthy controls, demonstrating that CRP concentrations are also affected in refractory epilepsy.

There is an increasing interest in AED-related inflammatory changes. The duration of AED therapy has been associated with the acceleration of atherosclerosis in patients with epilepsy (70% refractory and polytherapy, no classification of the syndrome), along with independent contributions of age, gender, and oxidative stress to the atherosclerotic process (Tan et al., 2009). The mean CRP concentration in patients who were taking enzyme-inducing drugs was twice as high as in patients taking noninducing drugs and probably because of the small sample size the difference was not statistically significant. The baseline concentrations were higher in patients with refractory epilepsy compared with healthy controls. In a previous study, mean levels between patients with epilepsy and controls were not different (Mintzer et al., 2009), but in the present study, the patients with XLE had fourfold and with TLE a ninefold increase compared with controls.

The epilepsy type was significantly associated with CRP concentrations; all five patients with elevated CRP-0h had TLE compared with none with XLE. Mesial TLE (MTLE) is the most commonly studied focal epilepsy. Refractory MTLE is also the most common indication for epilepsy surgery, and hippocampal atrophy seems to develop in the course of time (Salmenperä et al., 2005); the hippocampal volume loss can correlate with the number of generalized seizures, suggesting seizure-associated hippocampal damage (Briellmann et al., 2002). There is accumulating evidence from EEG, positron emission tomography, and magnetic resonance spectroscopy that MTLE is a network disease affecting also extrahippocampal structures (Wieser & ILAE Commission on Neurosurgery of Epilepsy, 2004). Patients with TLE have increased prevalence of generalized brain atrophy as well as cerebellar atrophy (Seidenberg et al., 2005). There is much less evidence of progressive damage in the extratemporal epilepsy. There are also data on seizure-induced (Alapirtti et al., 2009) and chronically increased production of IL-6 in therapy-resistant TLE (Liimatainen et al., 2009). In addition, highly proinflammatory plasma cytokine profile (high IL-6, low IL-1Ra, and low IL-1Ra/IL-1b ratio) was found without any evidence of increased production from peripheral blood mononuclear cells (Hulkkonen et al., 2004). However, the exact role of immune activation in disease progression in relation to TLE remains undetermined.

The seizure type was a major determinant of the CRP responses after the index seizure. In four of five patients with SGTCS, the index seizure CRP level increased, particularly after 3 h, whereas in partial seizures there was no change after index seizure. IL-6 is a major inducer of CRP (Mantovani et al., 2008). We have ample evidence suggesting the activation of IL-6 system after epileptic seizures in our previous studies, according to which the duration and extent of the seizures is associated with a more pronounced IL-6 response (Lehtimäki et al., 2004). In a previous study in patients with recent generalized tonic–clonic seizures, plasma IL-6 concentrations correlated with peripheral blood leukocyte counts and CRP; there was also evidence of activation of indicators of inflammation including CRP (Peltola et al., 2002). Our data suggest that generalized tonic–clonic seizures stimulate CRP production.

In line with baseline CRP level, older patients had significantly higher mean of all five CRP measurements during the baseline and 24 h measurements after the index seizure. Higher baseline CRP levels were also detected in those with older age at diagnosis. In addition, CRP level was higher in older controls than in younger controls. This is in line with results of previous large studies from general population as a well-known fact that CRP is higher in older people (Donato et al., 2008).

Experimental and human studies have shown that chronic inflammation may play a role also in the neurodegenerative process. There are data that link activation of inflammatory biomarkers such as IL-6 and total brain volume loss in patients with dementia (Engelhart et al., 2004) and in the general population (Jefferson et al., 2007). In nondemented children with Down syndrome, plasma levels of IL-6, CRP, soluble IL-6 receptor, soluble intercellular adhesion molecule-3, and soluble vascular cell adhesion molecule-1 were significantly higher than in control children as a possible early sign of central nervous system alterations leading many years later to cognitive deterioration and dementia (Licastro et al., 2005). The increase of IL-6 and CRP from children was Down syndrome was similar to that found in elderly patients with clinical Alzheimer's disease. As in a recent study, elevated CRP was noted in patients with epilepsy and at the same time increased intima media thickness at the wall of the common carotid artery (Tan et al., 2009); there may be increased risk of atherosclerosis either caused by AEDs or epilepsy itself. In refractory epilepsy, especially TLE, there is an increased risk of cognitive impairment (Nearing et al., 2007), and the frequency of SGTCS was the strongest predictor of decline (Thompson & Duncan, 2005); complex partial seizure frequency was associated with a decline in memory and executive skills but not in IQ. In future studies the role of CRP as a predictor of subsequent cognitive change should be evaluated also in patients with epilepsy.

There are several limitations to our study. Because our main focus was to evaluate seizure related changes, we have not gathered all potentially relevant information about the patients regarding all possible associations. A variety of factors have been recognized that are associated with minor elevation of CRP such as high blood pressure, or hypertriglyceridemia, exposure to air pollution, periodontitis or other low-grade inflammatory process, and poor physical conditioning (Kushner et al., 2006). We have no patient data about, for example, cholesterol level, blood pressure, or exercise habits. However, our patients showed no signs of clinical infections during registration.

The CRP levels in patients with refractory epilepsy higher than in healthy controls emphasize the association between inflammation and epilepsy. Our results suggest that the more severe seizure type (SGTCS) shows a stronger inflammatory response after an acute seizure. The usefulness and meaning of serial measurements of individual CRP responses in different epilepsy syndromes requires a larger study population.

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None of the authors has any conflict of interest to disclose.

Disclosure

We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

References

Alapirtti T, Rinta S, Hulkkonen J, Mäkinen R, Keränen T, Peltola J. (2009) Interleukin-6, interleukin-1 receptor antagonist and interleukin-1beta production in patients with focal epilepsy: a video-EEG study. *J Neurol Sci* 280:94–97.

Ballantyne CM, Hoogeveen RC, Bang H, Coresh J, Folsom AR, Chambless LE, Myerson M, Wu KK, Sharrett AR, Boerwinkle E. (2005) Lipoprotein-associated phospholipase A2, high-sensitivity C-reactive protein, and risk for incident ischemic stroke in middle-aged men and women in the atherosclerosis risk in communities (ARIC) study. *Arch Intern Med* 165:2479–2484.

Briellmann RS, Berkovic SF, Syngeniotis A, King MA, Jackson GD. (2002) Seizure-associated hippocampal volume loss: a longitudinal magnetic resonance study of temporal lobe epilepsy. *Ann Neurol* 51:641–644.

Commission on Classification and Terminology of the International League Against Epilepsy. (1981) Proposal for revised clinical and electroencephalographic classification of epileptic seizures. From the commission on classification and terminology of the international league against epilepsy. *Epilepsia* 22:489–501.

Donato AJ, Black AD, Jablonski KL, Gano LB, Seals DR. (2008) Aging is associated with greater nuclear NFkappaB, reduced IkappaBalpha, and increased expression of proinflammatory cytokines in vascular endothelial cells of healthy humans. *Aging Cell* 7:805–812.

Dziedzic T. (2006) Systemic inflammatory markers and risk of dementia. *Am J Alzheimers Dis Other Demen* 21:258–262.

Engelhart MJ, Geerlings MI, Meijer J, Kiliaan A, Ruitenberg A, van Swieten JC, Stijnen T, Hofman A, Witteman JC, Breteler MM. (2004) Inflammatory proteins in plasma and the risk of dementia: the Rotterdam study. *Arch Neurol* 61:668–672.

Hulkkonen J, Koskikallio E, Rainesalo S, Keränen T, Hurme M, Peltola J. (2004) The balance of inhibitory and excitatory cytokines is differently regulated in vivo and in vitro among therapy resistant epilepsy patients. *Epilepsy Res* 59:199–205.

Jefferson AL, Massaro JM, Wolf PA, Seshadri S, Au R, Vasan RS, Larson MG, Meigs JB, Keaney JF Jr, Lipinska I, Kathiresan S, Benjamin EJ, DeCarli C. (2007) Inflammatory biomarkers are associated with total brain volume: the Framingham heart study. *Neurology* 68:1032–1038.

Koskinen JO, Vaarno J, Meltola NJ, Soini JT, Hänninen PE, Luotola J, Waris ME, Soini AE. (2004) Fluorescent nanoparticles as labels for immunometric assay of C-reactive protein using two-photon excitation assay technology. *Anal Biochem* 328:210–218.

Kushner I, Rzewnicki D, Samols D. (2006) What does minor elevation of C-reactive protein signify? *Am J Med* 119:166.e17–166.e28.

Laihia JK, Koskinen JO, Waris ME, Jansen CT. (2005) Adaptation of the human skin by chronic solar-simulating UV irradiation prevents

ultraviolet-B irradiation-induced rise in serum C-reactive protein levels. *Photochem Photobiol* 81:654–658.

Lehtimäki KA, Keränen T, Huhtala H, Hurme M, Ollikainen J, Honkaniemi J, Palmio J, Peltola J. (2004) Regulation of IL-6 system in cerebrospinal fluid and serum compartments by seizures: the effect of seizure type and duration. *J Neuroimmunol* 152:121–125.

Licastro F, Chiappelli M, Ruscica M, Carnelli V, Corsi MM. (2005) Altered cytokine and acute phase response protein levels in the blood of children with Downs syndrome: relationship with dementia of Alzheimer's type. *Int J Immunopathol Pharmacol* 18:165–172.

Liimatainen S, Fallah M, Kharazmi E, Peltola M, Peltola J. (2009) Interleukin-6 levels are increased in temporal lobe epilepsy but not in extratemporal lobe epilepsy. *J Neurol* 256:796–802.

Lin HB, Yang XM, Li TJ, Cheng YF, Zhang HT, Xu JP. (2009) Memory deficits and neurochemical changes induced by C-reactive protein in rats: implication in Alzheimer's disease. *Psychopharmacology* 204:705–714.

Mantovani A, Garlanda C, Doni A, Bottazzi B. (2008) Pentraxins in innate immunity: from C-reactive protein to the long pentraxin PTX3. *J Clin Immunol* 28:1–13.

Mintzer S, Skidmore CT, Abidin CJ, Morales MC, Chervoneva I, Capuzzi DM, Sperling MR. (2009) Effects of antiepileptic drugs on lipids, homocysteine, and C-reactive protein. *Ann Neurol* 65:448–456.

Nearing K, Madhavan D, Devinsky O. (2007) Temporal lobe epilepsy: a progressive disorder? *Rev Neurol Dis* 4:122–127.

Peltola J, Laaksonen J, Haapala AM, Hurme M, Rainesalo S, Keränen T. (2002) Indicators of inflammation after recent tonic-clonic epileptic seizures correlate with plasma interleukin-6 levels. *Seizure* 11:44–46.

Pitkänen A, Sutula TP. (2002) Is epilepsy a progressive disorder? Prospects for new therapeutic approaches in temporal-lobe epilepsy. *Lancet Neurol* 1:173–181.

Salmenperä T, Kononen M, Roberts N, Vanninen R, Pitkanen A, Kälviäinen R. (2005) Hippocampal damage in newly diagnosed focal epilepsy: a prospective MRI study. *Neurology* 64:62–68.

Seidenberg M, Kelly KG, Parrish J, Geary E, Dow C, Rutecki P, Hermann B. (2005) Ipsilateral and contralateral MRI volumetric abnormalities in chronic unilateral temporal lobe epilepsy and their clinical correlates. *Epilepsia* 46:420–430.

Tan TY, Lu CH, Chuang HY, Lin TK, Liou CW, Chang WN, Chuang YC. (2009) Long-term antiepileptic drug therapy contributes to the acceleration of atherosclerosis. *Epilepsia* 50:1579–1586.

Thompson PJ, Duncan JS. (2005) Cognitive decline in severe intractable epilepsy. *Epilepsia* 46:1780–1787.

Wieser HG, ILAE Commission on Neurosurgery of Epilepsy. (2004) ILAE commission report. Mesial temporal lobe epilepsy with hippocampal sclerosis. *Epilepsia* 45:695–714.

Xu G, Zhou Z, Zhu W, Fan X, Liu X. (2009) Plasma C-reactive protein is related to cognitive deterioration and dementia in patients with mild cognitive impairment. *J Neurol Sci* 284:77–80.

The concentration of cell-free DNA in video-EEG patients is dependent on the epilepsy syndrome and duration of epilepsy

Tiina Alapirtti[1], Juulia Jylhävä[2], Jani Raitanen[3], Riikka Mäkinen[4], Jukka Peltola[1], Mikko A. Hurme[2,5], Suvi Liimatainen[1]

[1]Department of Neurology and Rehabilitation, Tampere University Hospital, Tampere, Finland; [2]Department of Microbiology and Immunology, University of Tampere, School of Medicine, Tampere, Finland; [3]School of Health Sciences, Finland and UKK Institute for Health Promotion, Tampere, Finland; [4]Medical Imaging Centre, Pirkanmaa Hospital District, Tampere, Finland, [5]The Laboratory Centre, Tampere University Hospital, Tampere, Finland.

Key words: Cell-free DNA; Focal epilepsy; Temporal lobe epilepsy; Extra-temporal lobe epilepsy; Duration of epilepsy

Abstract

Objective: Cell-free DNA (cf-DNA) is a marker of inflammation and cell death. The purpose of the present study was to analyse the role of cf-DNA as a putative biomarker in refractory epilepsy.

Methods: Baseline concentration of cf-DNA was measured in the serum of 51 carefully evaluated refractory epilepsy patients undergoing video-EEG monitoring. Epilepsy was classified based on seizure semiology, patient history and imaging findings. Majority of the patients (47) had focal epilepsy. The association of the concentration cf-DNA with different clinical determinants was analyzed. 250 healthy individuals served as control subjects.

Results: The mean baseline concentration of cf-DNA was lower in patients with extra temporal lobe epilepsy (XTLE) compared to control subjects (0.72 μg/ml vs. 0.80 μg/ml; $p = 0.001$). The concentration of cf-DNA between patients with temporal lobe epilepsy (TLE) and control subjects was not significant. The maximum concentration of cf-DNA after baseline measurement was significantly lower in patients with duration of epilepsy ≥ 18 years compared to those with duration of epilepsy < 18 years (0.022 μg/ml vs. 0.031 μg/ml; $p = 0.044$). The maximum concentration of cf-DNA was higher in patients with body mass index (BMI) ≥ 25 compared to those with BMI < 25 (0.004 μg/ml vs. 0.041 μg/ml; $p = 0.006$).

Discussion: The difference between the concentration of cf-DNA in patients with XTLE and control subjects strengthens the previous observations of the importance of epilepsy type with regard of different biomarkers.

Introduction

Focal epilepsies can be progressive disorders leading to permanent neuronal damage, worsening seizures, and cognitive decline.[1] Biomarkers for neuronal damage could be useful in identifying patients in danger of progressive neuronal damage in an earlier state. Cell-free (circulating) nucleic acids are compounds which are dispersed in body fluids. Cell-free DNA (cf-DNA) has shown inflammatory and degenerative properties in a variety of medical conditions. The concentration of cf-DNA increases in trauma, sepsis, myocardial infarction, transplantation, and diabetes mellitus among other.[2] Cf-DNA can activate the innate immune system and a strong correlation exists between the level of circulating nucleosomes, interleukin-6 (IL-6) and tumor necrosis factor α (TNF-α).[2] DNA release following cell death and active secretion of DNA from cells are theoretical major sources of cf-DNA.[2] Increased levels of cf-DNA have been measured in stroke and traumatic brain injury. [3,4] We have previously studied cf-DNA in epilepsy and observed increased concentration in focal epilepsy, especially in symptomatic epilepsy.[5] In the acute setting, cf-DNA levels correlated with the severity of the disease and the outcome in patients with traumatic brain injury and stroke.[3,4] The potential role of cf-DNA in neurological diseases is, however, not well established.

We have previously studied various markers of inflammation and neuronal damage in refractory epilepsy patients undergoing video-EEG evaluation.[6-9] The benefit of video-EEG is the possibility to exactly classify and evaluate the pre- and postictal effects of different seizure types and the duration of seizures on specific markers. In the present study we wanted to evaluate the baseline concentrations as well as the effect of acute seizures on the concentration of cf-DNA to better understand the significance of this specific marker and the association with various clinical determinants. Moreover, our aim was to increase the understanding of the complicated interplay between inflammatory and neurodegenerative processes in refractory epilepsy.

Material and methods

Fifty-one consecutive patients with refractory epilepsy admitted to the video-EEG monitoring unit of the Tampere University Hospital were included in the study. The study protocol was approved by the Ethics Committee of the Tampere University Hospital, and all the patients signed a written informed consent. The study protocol followed the guideline of Declaration of Helsinki and Uniform Requirements for manuscripts submitted to Biomedical journals.

All patients underwent continuous video-EEG monitoring lasting for four days for electroclinical characterization of their seizures as part of the routine clinical evaluation for possible epilepsy surgery. Ictal scalp recordings were obtained using synchronous digital video and 24 channel standard bipolar EEG. Electrodes were placed according to the International 10-20 System with additional mastoid and anterior cheek electrodes. All patients underwent a diagnostic brain MRI examination on a 1.5 (General Electric, Sigma HD, Milwaukee, Wisconsin, USA) or 3.0 (Siemens Healthcare, Magnetom Trio A Tim system 3T, Erlangen, Germany) Tesla machine. Seizures and epileptic syndromes were classified according to the International League Against Epilepsy (ILAE) diagnostic criteria.[10] Anticonvulsant medication was discontinued in a stepwise fashion during the monitoring by the evaluation of the staff epileptologist.

Localization of the seizure focus was recorded and seizures were categorized as simple partial (SPS), complex partial (CPS), secondarily generalized tonic-clonic seizures (SGTCS), or primary generalized tonic-clonic seizures (PGTCS). The first unequivocally verified seizure during the video-EEG monitoring was considered as the index seizure (IS). All other seizures after IS during the next 24 hours were also registered. Based on the findings in the video-EEG recordings and MRI, 23 patients had temporal lobe epilepsy (TLE), 24 patients had extratemporal lobe epilepsy (XTLE) (21 patients with frontal lobe, two with parietal lobe, and one with multilobar epilepsy), and four

patients had idiopathic generalized epilepsy (IGE). Seven patients were on monotherapy, 43 on polytherapy (the mean number of medications 2.4), and one patient had no medication. In addition to antiepileptic drugs, 4 patients were treated with vagus nerve stimulation. One female patient with right hippocampal sclerosis was monitored twice over an interval of 23 months. Seizure burden was measured by the total duration of seizures recorded during the 24h sampling period. Body mass index (BMI) was recorded.

Blood samples were collected at the onset of video-EEG recording and then daily in the morning until patients had their IS and at 3, 6, 12 and 24 hours after the IS. Last sample before the IS was the baseline sample. The samples were stored at -70 ^{o}C until analyzed. The concentration of circulating cf-DNA was measured directly in serum using a Quant-iT^{TM} DNA High-Sensitivity Assay kit and a Qubit® fluorometer (Invitrogen, Carlsbad, CA, USA) following the standard protocol according to the manufacturer's instructions. All the samples were analyzed in duplicate, and the mean of the two replicates was used as the final value. The third sample was analyzed if the difference between two values was more than 20%. 141 healthy female and 109 male served as control subjects.[11]

Statistical analyses

Descriptive analysis was used to outline the characteristics of the participants by scatterograms, frequencies, means and standard deviations. Due to the small sample size ordered logistic regression models (also known as proportional odds models) were used to test an association between concentration of cf-DNA at baseline (dependent variable) and patients' age, etiology of epilepsy, duration of epilepsy, seizure frequency, or BMI (independent variables). We used median as a cut point to divide patients' age, duration of epilepsy, seizure frequency, and BMI into two groups. In order to use ordered logistic regression models continuous cf-DNA was categorized into

quartiles. Each model was adjusted for age and gender, and the assumption of proportional odds (i.e. that regression coefficients are constant across levels of dependent variable) were tested for all models by the test of parallel lines.

The difference between the baseline and maximum concentration of cf-DNA after the baseline was calculated. Mann-Whitney U-test was used to compare the distributions of difference in patients' age, duration of epilepsy, seizure frequency and BMI. Kruskal-Wallis test was used in etiology of epilepsy.

The significance of all tests was set at 0.05 and data analysis was performed using IBM SPSS Statistics version 19.

Results

Clinical characteristics of the study population are presented in Table 1. The baseline concentrations of cf-DNA were dependent on the epilepsy syndrome. The mean concentration in the control population was 0.80 μg/ml, whereas in the patients with TLE the mean concentration was 0.82 μg/ml and in patients with XTLE 0.72 μg/ml. The difference between the patients with XTLE and control subjects was statistically significant ($p=0.001$) (Figure 1). In the ordered logistic regression subgroup analyses, other clinical determinants such as patients´ age, etiology of epilepsy, duration of epilepsy, or seizure frequency did not have an effect on the baseline concentration of cf-DNA. The concentration of cf-DNA was lower in female patients (0.71 μg/ml) compared to male patients (0.83 μg/ml; $p=0.022$). Similar difference was observed in control subjects; female controls had lower concentration of cf-DNA (0.76 μg/ml) compared to males (0.86 μg/ml; $p<0.001$).

Insert Table 1 about here

Insert Figure 1 about here

The effect of seizure on the concentration of cf-DNA was influenced by the duration of epilepsy and BMI (Table 2). When the maximal difference between the baseline and the peak concentration after IS was determined, there was no significant difference in all epilepsy patients (Figure 2). However, when different clinical determinants were included in the analysis, there was a significant difference ($p=0.044$) between patients with the duration of epilepsy less than 18 years (0.031 μg/ml) and those patients with ≥ 18 years of duration (0.022 μg/ml). The difference was also significant ($p=0.006$) in patients with BMI ≥ 25 (0.041 μg/ml) compared with those with BMI under 25 (0.004 μg/ml). The baseline concentration of cf-DNA did not differ between patients with BMI under 25 and those with BMI ≥ 25. Patients´ age, epilepsy syndrome, duration of epilepsy, or seizure frequency did not explain the changes of the concentration of cf-DNA at different time points after IS. The effect of seizure burden on the changes of the concentration of cf-DNA was not significant.

Insert Table 2 about here

Insert Figure 2 about here

Discussion

This study has two major findings. First, the mean baseline concentration of cf-DNA was lower in patients with XTLE compared to controls whereas there was no difference between patients with TLE and control subjects. The second finding addresses the duration of epilepsy and BMI as modifiers of the seizure related effects. In the present study, there was some indication of a difference between TLE and XTLE groups, although in the direct comparison the difference was not statistically significant. The inherent problem of acquiring large enough study population in this demanding setting further compromises the statistical analysis. The chronic epileptic process includes complex neuronal pathways leading to neurodegeneration, which is supported by the present finding of minor changes of cf-DNA in the patients with a long duration of the disease.[1]

Cf-DNA is associated with inflammation and neurodegeneration in a variety of human and experimental conditions.[2] The exact function of cf-DNA with regard to clinical disorders is not yet fully understood. It may either activate or reflect neuronal damage and inflammation, but on the other hand it has been suggested that it could also be involved with neuronal repair.[2]

The duration of epilepsy was a significant determinant on the seizure associated increase of the concentration of cf-DNA.[7-9,12] Patients with a shorter duration of epilepsy demonstrated a positive change, whereas practically no change was observed in patients with a longer history of epilepsy. This finding may be explained by the dynamic nature of the evolution of epilepsies. The fine intrinsic balance between the activation of neurodegeneration and restoration may change into a more indolent phase.[13]

BMI was a critical modulator of acute production of cf-DNA in epilepsy. There is some clinical data suggesting a role for BMI in association with cf-DNA production. In a recent study there was a positive correlation between BMI and cf-DNA in women indicating an increased risk of cardiovascular diseases.[14] According to the previous findings, it is probable that the increased cf-DNA in overweight patients is associated with excess cardiovascular morbidity.[14] The present findings may suggest the similar prognosis profile in overweight patients with refractory epilepsy, although there was no difference in the baseline concentration of cf-DNA with regard to BMI.

In our previous study, majority of patients with focal epilepsy had increased concentration of cf-DNA suggesting the active inflammatory and possible degenerative process in the epileptic brain.[5] CRP concentration changes were evaluated in a separate study with same video-EEG patient population. The results demonstrated increased CRP levels in patients with secondarily generalized

tonic clonic seizures emphasising the significance of the severity of the seizure in producing inflammatory acute phase reactions.[9] Although the seizure induced changes in the present study were not comparable to the previously studied markers (CRP, IL-6 and S-100), some interesting findings emerged.

The significance and the pathophysiological role of the decreased level of cf-DNA in epilepsy remain unclear. All the previous studies suggest the increased concentration of cf-DNA in acute neurological disorders and in patients with chronic epilepsy.[3-5] In addition, the majority of studies in patients with acute seizures suggest activation of inflammatory mediators. Seizures also increase the levels of leucocytes both in cerebrospinal fluid and blood.[15] It is possible that this activation of acute phase reaction might activate rapid consumption and downregulation of harmful mediators.[6] This theory might be supported by the previous observation in patients with recent seizures, in which - in addition to an increase of IL-6 after seizure - the levels of soluble IL-6 receptors (IL-6R) decreased postictally.[6] However, inflammatory pathways and effects of single inflammatory mediators are complex. For example IL-6 levels increase after seizures but on the other hand IL-6 may also exert proconvulsive effects.[16]

The limitation of this study includes the relatively small study population especially in terms of subgroup analysis. However, taken into consideration the practical difficulties performing a study in video-EEG settings the number of patients is comparable to other similar studies.

To conclude, the study demonstrated a significant difference on the concentration of cf-DNA in patients with XTLE compared to control subjects emphasizing the clinical significance of epilepsy syndrome on the production of this marker. Longer duration of epilepsy was associated with lower

increase of cf-DNA values after IS suggesting irreversible inflammatory profile in these patients. High BMI was associated with increased concentration of cf-DNA paralleling previous findings and possible poorer prognosis in overweight epilepsy patients.

Considering the complexity of the pathogenesis of epilepsy, it is unlikely that a single biomarker could solve the problems of predicting the final outcome. On the other hand, it is important to describe the individual features of a given biomarker with regard to seizure types, epileptic syndromes and different etiologies. In the future we need to address a group of biomarkers representing different brain and body compartments in the same way as has been demonstrated in patients with mild traumatic brain injury.[17] The pathophysiological explanation of the decrease and increase of the levels of cf-DNA remains unclear but neurodegenerative, pro- or anti-inflammatory and neuromodulative effects are possible.

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Conflict of interest

Jukka Peltola has received support from Cyberonics, Eisai, Schwarz-Pharma, Jazz Pharmaceuticals, GlaxoSmithKline, Medtronics, UCB Pharma, Janssen-Cilaq, and Pfizer. Suvi Liimatainen has received support from GlaxoSmithKline UCB Pharma, Lundbeck, and OctaPharma. Tiina Alapirtti, Juulia Jylhävä, Jani Raitanen, Riikka Mäkinen, and Mikko A. Hurme have no conflict of interest.

References

1. Pitkänen A, Sutula TP. 2002. Is epilepsy a progressive disorder? Prospects for new therapeutic approaches in temporal-lobe epilepsy. Lancet Neurol. 2002;1: 173-181.
2. Mittra I, Nair NK, Mishra PK. Nucleic acids in circulation: are they harmful to the host? J Biosci. 2012;37:301-312.
3. Campello Yurgel V, Ikuta N, Brondani da Rocha A, Lunge VR, Fett Schneider R, Kazantzi Fonseca AS, et al. Role of plasma DNA as a predictive marker of fatal outcome following severe head injury in males. J. Neurotrauma. 2007;24:1172-1181.
4. Tsai NW, Lin TK, Chen SD, Chang WN, Wang HC, Yang TM, et al. The value of serial plasma nuclear and mitochondrial DNA levels in patients with acute ischemic stroke. Clin. Chim. Acta. 2011;412:476-479.
5. Liimatainen SP, Jylhävä J, Raitanen J, Peltola JT, Hurme MA. The concentration of cell-free DNA in focal epilepsy. Epilepsy Res. 2013;105,:292-298.
6. Lehtimäki KA, Keränen T, Huhtala H, Hurme M, Ollikainen J, Honkaniemi J, et al. Regulation of IL-6 system in cerebrospinal fluid and serum compartments by seizures: the effect of seizure type and duration. J. Neuroimmunol. 2004;152:121-125.
7. Palmio J, Keränen T, Alapirtti T, Hulkkonen J, Mäkinen R, Holm P, et al. Elevated serum neuron-specific enolase in patients with temporal lobe epilepsy: a video-EEG study. Epilepsy Res. 2008;81:155-160.

8. Alapirtti T, Rinta S, Hulkkonen J, Mäkinen R, Keränen T, Peltola J. Interleukin-6, interleukin-1 receptor antagonist and interleukin-1beta production in patients with focal epilepsy: A video-EEG study. J. Neurol. Sci. 2009;280:94-97.
9. Alapirtti T, Waris M, Fallah M, Soilu-Hänninen M, Mäkinen R, Kharazmi E, et al. 2012. C-reactive protein and seizures in focal epilepsy: a video-electroencephalographic study. Epilepsia. 2012;53:790-796.
10. Commission on Classification and Terminology of the International League Against Epilepsy: Proposal for revised classification of epilepsies and epileptic syndromes. Epilepsia. 1989;30:389-399.
11. Jylhävä J, Lyytikäinen LP, Kähönen M, Hutri-Kähönen N, Kettunen J, Viikari J, et al. A genome-wide association study identifies UGT1A1 as a regulator of serum cell-free DNA in young adults: The Cardiovascular Risk in Young Finns Study. PLoS One. 2012;7:e35426.
12. Palmio J, Peltola J, Vuorinen P, Laine S, Suhonen J, Keränen T. Normal CSF neuron-specific enolase and S-100 protein levels in patients with recent noncomplicated tonic–clonic seizures. J. Neurol. Sci. 2001;183:27-31.
13. Pitkänen A, Nissinen J, Nairismägi J, Lukasiuk K, Gröhn OH, Miettinen R, et al. Progression of neuronal damage after status epilepticus and during spontaneous seizures in a rat model of temporal lobe epilepsy. Prog. Brain Res. 2002;135:67-83.
14. Jylhävä J, Lehtimäki T, Jula A, Moilanen L, Kesäniemi YA, Nieminen MS, et al. Circulating cell-free DNA is associated with cardiometabolic risk factors: the Health 2000 Survey. Atherosclerosis. 2014;233:268-271.

15. Peltola J, Laaksonen J, Haapala AM, Hurme M, Rainesalo S, Keränen T. Indicators of inflammation after recent tonic-clonic epileptic seizures correlate with plasma interleukin-6 levels. Seizure. 2002;11:44-46.
16. Kalueff AV, Lehtimäki KA, Ylinen A, Honkaniemi J, Peltola J. Intranasal administration of human IL-6 increases the severity of chemically induced seizures in rats. Neurosci. Let. 2004;365:106-110.
17. Mondello S, Schmid K, Berger RP, Kobeissy F, Italiano D, Jeromin A, et al. The challenge of mild traumatic brain injury: role of biochemical markers in diagnosis of brain damage. Med. Res. Rev. 2014;34:503-531.

Table 1. Clinical characteristics of patients in video-EEG

	TLE	XTLE	IGE
No. of patients	23	24	4
Male/female	12/11	11/13	0/4
Age, mean (range)	40.0 (20-58)	30.0 (16-52)	32 (27-45)
BMI, mean (±S.D.)	25.9±3.96	25.0±4.39	26.2±3.51
Mean duration of epilepsy, years (range)	23.2 (2-56)	19.3 (1-52)	18.3 (6-32)
Mean seizure frequency/month[a] (range)	8.5 (0.5-30)	56.4 (0.5-240)	1.9 (9.5-4)
Mean number of AEDs	2.17	2.58	2.25
Patient on mono-/polytherapy	4/19	2/21	1/3
MRI findings			
Normal	4	12	3
HS	13	-	
Cortical dysplasia	3	6	
Other	3	6	1
Index seizure type			
SPS	1	3 (1 SPSE)	
CPS	19	17	
SGTCS	3	3	
PGS	-	-	4
VNS/earlier epilepsy surgery	2/0	2/1	-/-

TLE = temporal lobe epilepsy; XTLE = extratemporal lobe epilepsy; IGE = idiopathic generalized epilepsy; AED = antiepileptic drug; CPS= complex partial seizure; BMI = body mass index =weight kg/height m^2; HS = hippocampal sclerosis; PGS = primary generalized tonic-clonic seizure; SGTCS = secondary generalized tonic-clonic seizure; SPS = simple partial seizure; SPSE = simple partial status epilepticus; VNS = vagus nerve stimulation.

[a] During the last year

Table 2. Difference between maximum and baseline concentration of cf-DNA

	n	Median	Mean	Std. error	P
Patients with epilepsy	50	0.007	0.025	0.021	
Age					0.79 [1]
≤32	25	0.005	0.036	0.040	
>32	25	0.010	0.014	0.013	
Epilepsy syndrome					0.73 [2]
XTLE	23	0.010	0.046	0.038	
TLE	23	0.001	0.004	0.018	
IGE	4	0.009	0.026	0.110	
Lateralization of seizure					0.43 [2]
Left	21	0.002	0.009	0.013	
Right	14	-0.004	-0.008	0.022	
Other	15	0.040	0.078	0.064	
IS type					0.49 [1]
Generalized	6	0.014	0.023	0.048	
Partial	44	0.003	0.025	0.023	
Duration of epilepsy					0.044 [1]
≤18	24	0.027	0.031	0.021	
>18	25	-0.005	0.022	0.037	
Seizures per month last year					0.13 [1]
≤10	26	-0.005	0.004	0.022	
>10	24	0.019	0.047	0.036	
Duration of index seizure (sec)					0.077 [1]
≤65	26	0.019	0.061	0.034	
>65	24	-0.005	-0.014	0.021	
Seizure burden, all seizures (including IS, sec)					0.50 [2]
10-99	17	0.005	0.011	0.025	
100-249	17	0.010	-0.013	0.019	
250+	16	0.003	0.080	0.055	
BMI (kg/m^2)					0.006 [1]
<25	22	-0.009	0.004	0.043	
≥25	28	0.021	0.041	0.016	

Notes: XTLE = extratemporal lobe epilepsy; TLE = temporal lobe epilepsy; IGE = idiopathic generalized epilepsy; IS = index seizure; BMI = body mass index.
[1] Mann-Whitney test.
[2] Kruskal-Wallis test.

Figure 1

Baseline and mean concentration of cf-DNA in patients with extra-temporal lobe epilepsy (XTLE), patients with temporal lobe epilepsy (TLE) and control subjects (µg/ml)

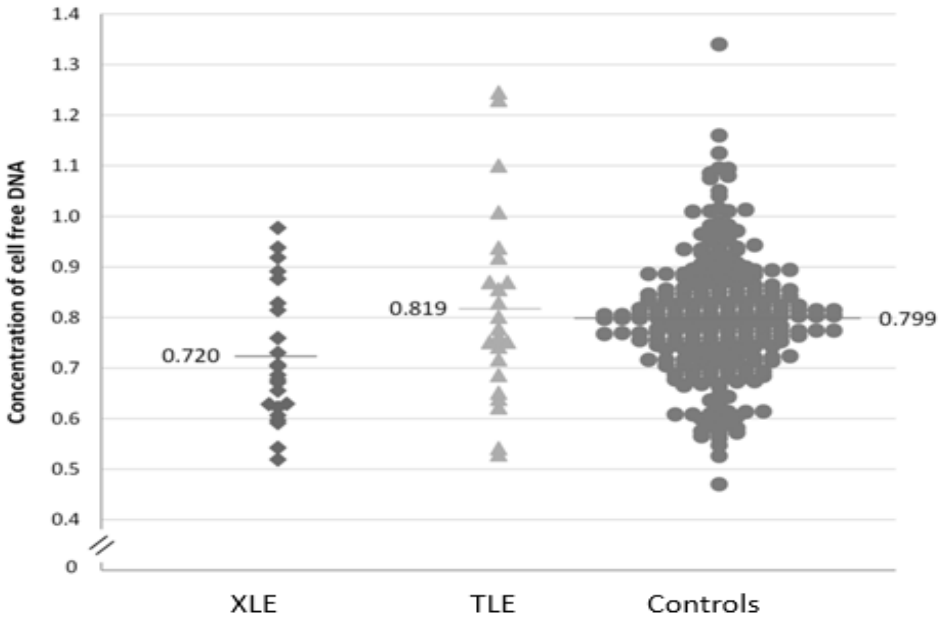

Figure 2

Maximal and mean difference between the baseline and peak concentration of cf-DNA after index seizure (IS) (µg/ml) in study patients