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The Possibility of Using Serum Concentrations of the Tumor Necrosis Factor-Alpha As a Biomarker in Mesial Temporal Lobe Epilepsy Associated With the Human Herpes Virus Neuroinfections

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Abstract

In this study, mesial temporal lobe epilepsy (MTLE) is shown to be associated with human herpes virus (HHV) neuroinfections. We also demonstrate that the epileptic process is associated with an inflammatory reaction, and that the proinflammatory cytokine, the tumor necrosis factor-alpha (TNF-α) is able to potentiate the reproduction of the herpes viruses. The study group (SG) included 43 patients between 16 and 60 years with MTLE and HHV neuroinfections, diagnosed according to the PCR of the cerebrospinal fluid (CSF), serum or abnormal serum/CSF IgG ratio. The control group (CG) included 20 patients of similar age with MTLE, but without the HHV neuroinfections. The concentration of TNF-α in the serum was determined by enzyme-linked immunosorbent assay ("VektorBEST" RF; N=0-50 pg/ml). Patients of the SG had high concentrations of TNF-α in serum (288±44.7 pg/ml), that were significantly higher than in the CG ($p<0.05$; $Z<Z0.05$). Serum concentrations of TNF-α greater than 100 pg/ml were associated with the severe general condition of the patients, more severe epileptic syndrome, a long history of illness, deep organic brain damage, low sensitivity to anticonvulsant drugs, overall with a poor prognosis. In patients with MTLE and HHV neuroinfections marked systemic inflammatory response syndrome was noted, which affected the severity of the symptoms in the patient. TNF-α, therefore, can be used as a biomarker for an objective assessment of the severity and prognosis of the disease in patients with MTLE induced by HHV. IJBM 2012; 2(1):16-25.

Key words: *TNF-α, mesial temporal lobe epilepsy, herpes virus, biomarker*

Introduction

The introduction of the biomarkers into clinical practice to objectively assess the severity of the patients and predict the future course of the disease, significantly improved the quality of medical services for patients with certain severe neurodegenerative diseases.

In 2001, an NIH working group standardized the definition of a biomarker as "a characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes or pharmacologic responses to a therapeutic intervention" and defined the types of biomarkers. More recently, by employing the biochemical analysis of blood serum and cerebrospinal fluid, as well as referring to neuroimaging and neurophysiological studies, a number of biomarkers with diagnostic sensitivity were identified, with respect to the progression of neurodegenerative diseases. This approach has improved the existing algorithms for diagnosis and has revealed new targets for the influence of drugs. Particularly, the determination of the concentration of beta-amyloid and phosphorylated tau protein in the serum and cerebrospinal fluid is used to verify the diagnosis and assessment of the severity of

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neurodegeneration and rational prediction of Alzheimer's disease [2]. In amyotrophic lateral sclerosis, the determination of serum concentrations of tyrosine [3], glutamate [4], fibronectin [5], hyaluronic acid [6], interleukin-6 [7], transforming growth factor beta-1 [8] and monocyte chemotactic protein 1-alpha [9] were suggested, for the same purpose.

Although, MTLE is a typical neurodegenerative disease with progressive development, thus far no reliable laboratory parameters have been identified for the assessment of the activity of the degenerative process in the nervous tissue, in patients with this disease.

The search for biomarkers for MTLE is one of the major challenges of modern neuroscience, as estimating the severity of the patient condition based on the number and duration of seizures or the results of an electroencephalographic (EEG) examination has not always given sufficient results.

Recently, some important scientific research papers were published regarding the etiologic role of herpes viruses, mainly herpes virus type 6 (human herpes virus type 6, HHV-6) in patients with MTLE, which has been one of the most significant achievements. This is confirmed not only using the amazing correlation between the zones of typical reproduction of these viral agents in the CNS and the localization of epileptic foci in temporal lobe epilepsy, but also by identifying the DNA and antigens of HHV-6 by using PCR, DNA- hybridization in situ, as well as by using immunohistochemistry in the biopsy of the hippocampus and median parts of the temporal lobe in patients with MTLE and hippocampal sclerosis [10, 11, 13, 14]. Recently, the research team led by Fotheringham J [15], made a fundamental discovery of a specific mechanism of virus-induced epileptogenesis and neurodegeneration in the case of persistent HHV-6 neuroinfections. The HHV-6-mediated inhibition of the expression of the glutamate transporter EAAT-2 of astrocytes was proven, which could be the cause of the increasing release of the excitatory glutamate that can produce the proepileptogen effect by inducing glutamatergic excitotoxicity.

To achieve success in identifying suitable laboratory biomarkers of temporal lobe epilepsy, we find the latest discoveries in the field of Neuroimmunology, indicating that the temporal epileptic process is closely linked with the development of the inflammatory reaction in the brain tissue [16-18], which corresponds to the recent reports regarding the etiologic role of the herpes virus infection in the induction of temporal epilepsy. Moreover, signs of cerebral inflammation were also found in Alzheimer's disease [2, 19, 20], Parkinson [21, 22] and amyotrophic lateral sclerosis [23], which suggests the inflammatory nature of human neurodegenerative diseases [24].

In light of the above arguments, a suitable biological marker would be assessed to indicate the intensity of the cerebral inflammation and reproductive activity of the herpes virus, persisting in the median portions of the temporal lobes of the brain during virus-induced temporal lobe epilepsy. The tumor necrosis factor alpha (TNF-α) may be a very good indicative biomarker in temporal lobe epilepsy. This protein, with a molecular mass of 26 kDa of monocytic origin [9, 19], is a potent master-cytokine that triggers the production of other pro-inflammatory substances with the development of an expanded clinical and laboratory pattern of inflammation in the body in response to infectious agents, damage or other stressors [27]. The results from several studies indicate the elevated concentrations of this cytokine in the cerebrospinal fluid and serum of patients with epilepsy and other neurodegenerative diseases [16, 17].

TNF-α is known to increase the production of proinflammatory interleukins, chemokines, nitric oxide, eicosanoids, oxygen free radicals, metalloproteinases and other aggressive enzymes [28]. Theoretically, the concentration of the universal proinflammatory mediators such as TNF-α in the cerebrospinal fluid of patients with temporal lobe epilepsy, characterizes the activity of the persistent inflammatory response in the brain which mediates the development of progressive neurodegenerative changes. Therefore, TNF-α appears to be a promising biomarker for monitoring. Activated microglia, particularly, found in the area of the epileptic focus (its cells are derivatives of the monocytes), are an important producer of the multifunctional pro-inflammatory mediator during temporal epilepsy [18, 29]. However, the determination of TNF-α concentration in the cerebrospinal fluid is associated with many technical difficulties (including a trauma due to lumbar puncture) as well as the prejudicial attitude of some patients about these diagnostic manipulations.

Although, TNF-α penetrates poorly from the blood serum into the cerebrospinal fluid, and vice versa [25], a sharp increase in the permeability of the blood-brain barrier in epileptic patients (particularly refractory forms of the disease) allows us to reasonably consider that the TNF-α levels in the serum may also characterize the state of cerebral pathology in patients with the virus-induced epileptic syndrome. Moreover, on entering into the blood serum from the different inflammation foci, TNF-α can increase the permeability of the blood-brain barrier, thereby increasing their cerebral effects [28].

It has been established that on entering the blood and cerebrospinal fluid, the proinflammatory cytokines, including TNF-α, produced in different compartments of the human body, can produce a direct proconvulsant effect on the neurons and increase the sensitivity of the nerve cells to other proconvulsant agents, and thus reduce the viability of the neurons under stress [30]. The latter effect may be associated with the property of TNF-α to induce apoptosis in the infected, transformed and damaged cells [28]. First, we describe the proconvulsant effects of the proinflammatory cytokines in the analysis of the clinical experience of high-dose recombinant interferon alpha in Oncology and Hepatology, as epileptic paroxysms are typical side effects seen in many patients on long-term treatment [31]. In the future, such ideas can be developed in experimental and clinical study, as well as the consequences of systemic inflammatory response syndrome (SIRS), which occurs in patients with sepsis, polytrauma, burn disease, and certain other pathological conditions [32]. The studies of the febrile seizures in infants and their effect on the risk of the further development of temporal lobe epilepsy have given impetus to the research of pro-epileptogenic properties of proinflammatory cytokines [33, 34]. Moreover, results of experimental and clinical studies directly devoted to the study of proconvulsant effects of TNF-α have been

published. In particular, Kirkman N et al., have shown in an experimental model of virus-induced epilepsy that the injection of the murine encephalomyelitis virus increases the production of the proinflammatory cytokines TNF-α and IL-6 with the development of the inflammatory reaction in situ that is associated with the risk of increasing the formation of convulsions. Moreover, the experimental mice which had knock-out TNF-α and IL-6 genes were unable to generate seizures in response to the development of neuroinfections [35].

Galic M et al. showed that a significant increase in the convulsive readiness in the experimental animals was observed after a parenteral injection of the bacterial lipopolysaccharide, which causes the development of the systemic inflammatory process, with a sharp increase in the TNF-α concentration in the serum. [36]. Today, TNF-α is considered as an intermediary between the extracerebral inflammation and the induction of the inflammatory response in the brain, in response to the proconvulsant injection.

Moreover, Riazi K. et al. demonstrated that the induction of the inflammation in the intestines of laboratory animals by an injection of the bacterial lipopolysaccharide had promoted an increase in the tendency to form convulsions in response to the injection of several proconvulsants. Besides, in these rats a more clearly manifested inflammatory reaction was observed in the brain tissue along with precise symptoms of a concomitant neurodegenerative process. The parenteral injection of TNF-α produced a similar result in another group of animals. This suggests that the proinflammatory cytokines, particularly TNF-α, are the intermediaries between the development of a local inflammation in the intestine and a subsequent initiation of the inflammatory process in the brain. The results of this study demonstrate a close link between the inflammatory lesions of various locations and the tendency to induce the generation of convulsive paroxysms, and in particular, it may explain the increased seizures in epileptic patients during various infectious episodes [37].

Crespel A et al. in temporal lobe biopsy performed in 18 patients with temporal epilepsy and median hippocampal sclerosis, have found the activation of the intracellular expression of the proinflammatory intermediary, nuclear factor κV, and its effects were found to be associated with TNF-α. In control brain samples obtained from people who do not suffer from seizures, similar changes were noted. The results of the study demonstrated that in the hippocampal tissue of patients with temporal epilepsy, a chronic inflammatory process, mediated by an increased expression of nuclear factor κV, is present, which leads to the formation of the state of high alert to the generation of repeated seizures [38].

Thus, the concentration of the TNF-α in the cerebrospinal fluid and serum may be regarded not only as an indicator of cerebral inflammation activity, but also as a factor determining the severity of the condition of a patient with temporal epilepsy by a direct or indirect effect on the epileptogenesis and the process of neurodegeneration.

The group of foreign researchers under the leadership of Simon C., in their study of herpes virus infections, has found that the promoter of the genome of these viruses is closely associated with the signal transduction pathways, which are activated by the TNF-α. This fact explains the potentiating effect of TNF-α on the reproduction of the herpes viruses [39]. Therefore, it is not surprising that herpes infections are characterized by a sharp increase in the concentration of TNF-α in the serum and the subsequent reduction of some cytokines, particularly, gamma interferon, which is involved in the reactions of cytotoxicity at the destruction of virus-infected cells [40, 41]. Thus, TNF-α is a potent factor that not only enhances the inflammatory response in the CNS, but also mediates the increase in the reproduction of the herpes viruses. This pro-inflammatory mediator could affect the main pathogenetic mechanism of temporal lobe epilepsy as well as the potential etiological factor of the disease.

The results of the genetic and population studies of temporal lobe epilepsy in humans indicate the genetically determined propensity to excessive induction of the active inflammatory processes in response to different triggers, including viral agents. It is based on both the abnormally high production of the proinflammatory mediators and the failure of the anti-inflammatory components of the system, which normally control the intensity of the inflammatory reaction on the principle of negative feedback. Van Gassen K et al. found abnormalities in the genes of the innate immunity cells obtained on autopsy of the affected brain tissue of patients with temporal epilepsy. The most significant violations were found in the study of the expression of the glial chemokine CCL3 and CCL4 genes, the activity of which was ten times higher than that of the control data. These immune mediators, whose production is mediated by the effects of TNF-α, as commonly known, can raise the excitability of the neurons by affecting the specific membrane receptors. Among other violations detected, the abnormalities in the genes of the neuropeptides, the chaperones and the ubiquitin-proteasome system whose functioning is also closely related to TNF-α [42] should be noted. Besides this, Ishizaki Y et al. performed a comparative genetic study on 225 healthy people and 249 patients with febrile seizures, which are associated with an increased risk of temporal lobe epilepsy in the future. In the study group patients compared to the control group patients ($p=0.014$ and 0.013, respectively) a significant predominance of specific alleles of the anti-inflammatory cytokine IL-10 was noted, which induce a lower level of the production of the neurotransmitter. This fact anticipates the availability of the genetic propensity to implement a very active inflammatory response to the infectious agents and other factors [43]. IL-10 is known to produce antagonistic effects when compared with TNF-α; therefore, the low production of this cytokine may create preconditions for the development of a systemic inflammatory response syndrome, mediated by the latter.

Choi J et al. in their population-based study showed that genetically determined increased susceptibility of the organism to the development of inflammation correlated with a significantly higher risk of temporal lobe epilepsy during postnatal ontogenesis [44].

Finally, the long-term maintenance of high concentrations of the TNF-α in the serum can significantly impair the general condition of the patient, particularly inducing complications such as progressive weight loss (up to cachexia) [45], osteoporosis [46], myomalacia [47], chondrodystrophy [48], hyperthermia [49], anemia [50], anorexia [51], pain [52] and depression [53]. These

pathological processes induced by TNF-α can significantly affect human health, particularly the quality of life, and even be the cause of disability. Thus, the level of the serum TNF-α allows us to estimate not only the severity of the cerebral pathological process, but also the depth of the breach of the general condition of the patient, which is defined by the severity of the epileptic syndrome with related neurological deficits and the influence of the immune deficiency and systemic inflammatory response.

The purpose of this research, therefore, is to investigate the possibility of using serum concentrations of TNF-α as an informative biomarker of the severity and prognosis of the mesial temporal lobe epilepsy associated with herpes neuroinfections.

Materials and Methods

The study group (SG) included 43 patients aged 16 to 69 years with the MTLE in the form of complex partial epilepsy with primary and secondary generalized tonic-clonic seizures. All the patients have also had herpes viruses verified by PCR diagnosis of the cerebrospinal fluid and the blood serum, as well as comparative serological studies on the anomalous ratio of the serum and cerebrospinal fluid antibodies. The control group (CG) included 20 patients with the MTLE without HHV neuroinfections.

The severity of the epilepsy was determined by the number of seizures during one month and the results of the EEG in the interictal period, taking into account the number of serial episodes of paroxysmal and the epileptic status. The refractoriness to the anticonvulsant was assessed by the number of changes of the anticonvulsants for the period of the history of the disease, the level of doses of the anticonvulsants administered, as well as the cases of the combination therapy. Osteoporosis was verified using the results of the X-ray or ultrasound bone densitometry, and chondrodystrophy based on the x-ray examination or MRI of the spine. The reagents and equipment were used for PCR analysis by "DNA-Technology" (Moscow, Russian Federation). The serologic tests for measuring the concentration of the IgG antibodies to the HHV in the blood serum and cerebrospinal fluid were performed by ELISA, using the reagents "VECTOR-BEST" (Novosibirsk, Russian Federation). The types of HHV identified are presented in Table 1. The concentration of TNF-α in serum was determined by ELISA method using reagents "VECTOR-BEST" (Novosibirsk, Russian Federation).

Statistical analysis. Structural and comparative analyses were performed using the Microsoft Excel software. The data obtained were processed using the variation statistics method for the student's t-test with calculation of confidence indicators of the probability *p* (parametric test) and the number of characters Z (by Yu. Urbach, nonparametric test). On analysis, the distribution of the variable calculated frequencies of individual values, mean, standard error of the mean, Student's t-test, confidence level, and the level of significance *p* was calculated. The difference was considered reliable when $p<0.05$ and $Z<Z0.05$. The method of linear correlation with the calculation of the coefficient *r* was used to assess the relationship between the parameters studied. The $r>0.3$ was assessed.

Results

The average concentrations of TNF-α in the serum of the SG patients were almost six times higher than upper normal (288.1±44.7 pg/ml). This indicates the presence of the systemic inflammatory response syndrome in the patients. The average concentrations of the TNF-α were significantly higher in the SG patients than in the CG patients ($p<0.05$; $Z<Z0.05$) (Fig. 1). This fact indicates that the reactivated herpes neuroinfection was associated with a more pronounced proinflammatory mediator production when compared with the non complicated epileptic process, according the latest data, which was also based on the local inflammatory response. The values of the parameters in the SG patients varied from 0.6 to 703.3 pg/mL (95% CI: 198.7-377.5) (Table 2). Moreover, the average frequency of the epileptic seizures and duration of the disease were higher in the subgroup of patients with high serum concentrations of TNF-α (Fig. 2 and 3).

These data enable us to consider HHV as the cause of the abnormally increasing TNF-α synthesis in patients with MTLE. In turn, TNF-α is the significant risk factor for the systemic inflammatory response syndrome, which can potentially contribute to the enhanced reproduction of viral agents (positive feedback), and potentiate epileptic paroxysms due to a destabilizing effect on the bioelectric activity of the neurons, causing an increase in the permeability of the blood-brain barrier, and a decrease in the resistance of the cells of the central nervous system to edema and ischemia, and thus worsen the patient's general condition due to a number of adverse systemic effects.

Thus, we found that MTLE, associated with HHV neuroinfections, is accompanied by a significantly more pronounced inflammatory response than a similar form of epilepsy without the reproduction of the herpes virus agents in the CNS.

To assess whether the differences in the serum concentration levels of TNF-α in the patients of the SG and CG groups are a cause for the differences in the severity of the condition of the patients and in the disease prognosis and sensitivity to the recommended medications, we performed a structural analysis of the results (Fig. 4). The structural analysis revealed that 30% of the cases (13 patients) in the SG showed a high concentration of this neurotransmitter in the serum, and 23% (10 patients) revealed the maintenance of the cytokine above 100 pg/ml. A positive correlation was found between the serum levels of TNF-α and the severity of the epilepsy, particularly, with the frequency of the seizures reported ($r=0.45$), with the number of changes in the anticonvulsant medication during the history of the disease ($r=0.54$), characterizing the degree of refractoriness to anticonvulsant drugs, as well as the duration of the disease in months ($r=0.38$), with the volume of brain tissue damage on MRI-tomograms ($r=0.41$), which was determined by the number of lesions or the severity of atrophy of the brain tissue, mainly the temporal lobes. At the same time, no association was found between the concentrations of the TNF-α in the

Table 1

Data of clinical, laboratory and instrumental studies in the SG patients with serum levels of TNF-α more than 100 pg/mL

Patient, age	Frequency of seizures	MTLE duration	Type of virus infection	Neurological status	Brain MRI data	EEG data	TNF-α level (pg/mL)
Patient B., 18 yr.	Generalized tonic-clonic seizures, 2-3 times a week, the tendency to seriality, 4 status epilepticus docu-mented in anamnesis	18 yr.	HSV-1	Right-sided hemiparesis, ataxia, cognitive impairments.	Gross encephalomalacia and gliosis in the left temporal lobe, the right maxillary sinus cyst	Conglutination in the fronto-temporal leads	216.1
Patient Z., 16 yr.	Generalized clonic-tonic and partial seizures, 2-3 times a week	1.5 yr.	HHV-6	Chronic fatigue syndrome	Selective temporal lobe atrophy with the initial events of hippocampal sclerosis	Spike-wave complexes in the left temporal lobe	178.4
Patient Ch., 35 yr.	Generalized tonic-clonic and partial seizures, from several a day to 1-2 times a month, the tendency to seriality, 2 status epilepticus docu-mented in anamnesis	10 yr.	EBV + HHV-6	Mixed ataxia, cognitive impairments	Multiple foci of demyelination in the temporal and parietal lobes, the focus of gliosis in the projection of the anterior horn of the right lateral ventricle	Conglutination in the temporal leads	121.4
Patient K., 16 yr.	Generalized tonic-clonic and partial seizures, 2-3 times a month	12 yr.	HSV-1	Chronic fatigue syndrome, multiple disembriogenetic stigmas	Selective temporal lobe atrophy with the initial events of hippocampal sclerosis. Poorly differentiated convexital subarachnoid space	Slow waves, alternating with complexes of the peak-slow wave in the temporal leads	114.2
Patient V., 22 yr.	Generalized tonic-clonic seizures, almost daily, three status epilepticus documented in anamnesis	2 yr.	HHV-6	Mixed ataxia, bilateral pyramidal insufficiency, olfactory hallucinations, cognitive impairments	Selective temporal lobe atrophy with the initial events of hippocampal sclerosis. Basilar Impression.	Paroxysmal bilateral short-term acute flare-up of the polymorphic activity	277.6
Patient B., 43 yr.	Generalized tonic-clonic and partial seizures, 2-4 times a month	25 yr.	HSV-1 + EBV	Mixed ataxia, cognitive impairments	Multiple foci in the transition zone between the white and gray matter of the cerebral hemi-spheres (cerebral vasculitis). Polisinusit.	Single atypical spike-wave complexes	254.2
Patient G., 30 yr.	Generalized tonic-clonic and partial seizures, 3-4 times a month and more, cases of serial seizures	8 yr.	HHV-6	Bilateral pyramidal insufficiency, extrapyramidal disorders, coprolalia, cognitive impairment	Hydrocephalus, residual effects after cryosurgery of the amygdaloid nucleus.	Conglutination in the temporal leads	693.7
Patient K., 31 yr.	Generalized tonic-clonic and partial seizures, almost daily, 3 status epilepticus documented in anamnesis	29 yr.	HHV-6	Mixed ataxia, cognitive impairment, personality change	Hydrocephalus, temporal lobe atrophy, hippocampal sclerosis, small foci of demyelination in the temporal and frontal white matter	Conglutination in the temporal leads	702.9
Patient M., 21 yr.	Generalized tonic-clonic and partial seizures, 1-2 times a month	8 yr.	EBV + HHV-6	Left-sided pyramidal insufficiency, intracranial hypertension	Hydrocephalus, temporal lobe atrophy, hippocampal sclerosis	Sharp-and -slow wave complexes, peak-wave	144.9
Patient S., 49 yr.	Generalized tonic-clonic and partial seizures, 1-2 times a month, cases of serial seizures	14 yr.	EBV	Intracranial hypertension, cognitive impairment	Hydrocephalus, selective temporal lobe atrophy, hippocampal sclerosis, small foci of demyelination in the temporal white matter	Paroxysmal bilateral short-term acute flare-up of the polymorphic activity	342.9

Table 2
Results of statistical analysis in the groups

Group	X	m	M	CI 95%	min	max
SG (n=43)	288.1	44.7	42.5	198.7-377.5	0.6	703.3
CG (n=20)	82.2	20.1	24.3	42.0-102.3	0.8	158.2

Figure 1
Average serum concentrations of TNF-α (pg/ml) in patients of study and control groups

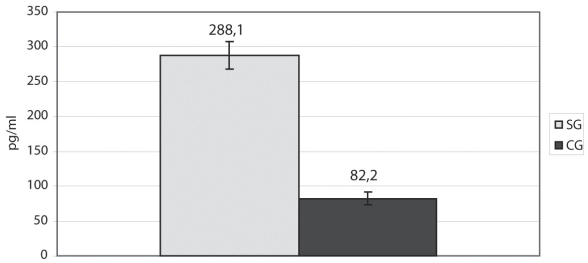


Figure 2
The average frequency of epileptic seizures in month in patients of the study group with high and low serum concentrations of TNF-α

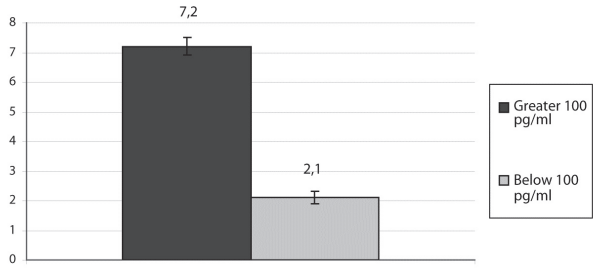


Figure 3
Disease duration (years) in patients of the study group with high and low serum concentrations of TNF-α

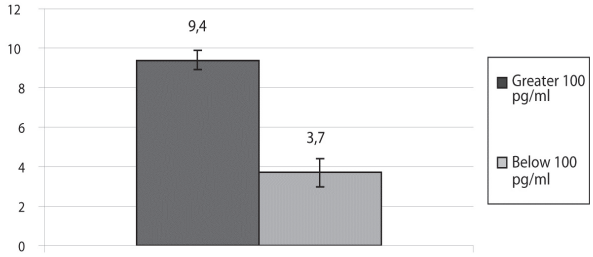
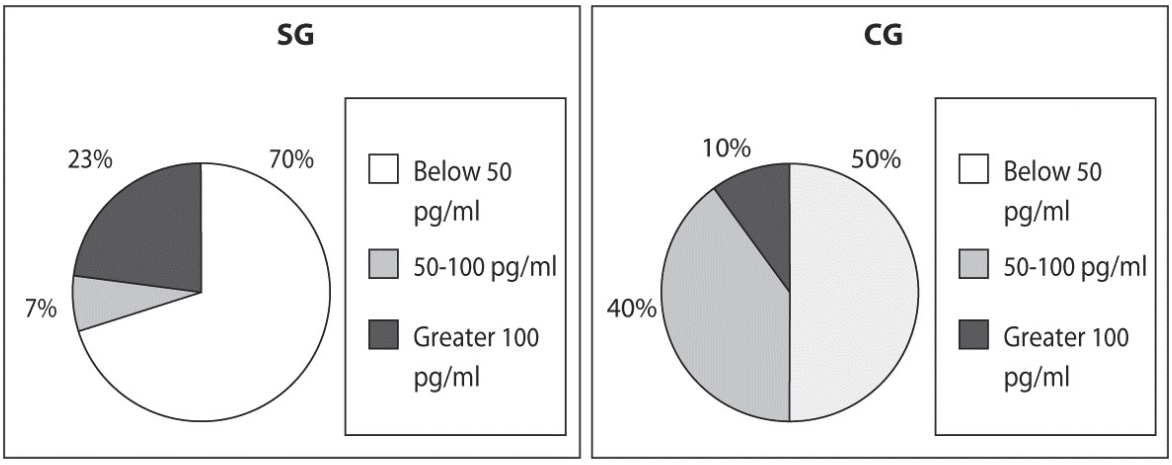


Figure 4
The results of structural analysis in the study and control groups in terms of the serum concentration of TNF-α



serum and the type of seizures, and the type of herpes virus, as well as the method of identification of the herpes agent. We did not evaluate the viral load caused by the herpes neuroinfections, although the reproductive activity of HHV-6 in the saliva was significantly higher in the patients with high serum concentrations of TNF-α ($p<0.05$; $Z<Z0.05$).

The known systemic effects of TNF-α were investigated separately. Thus, chronic hyperthermia was found twice as often, weight loss and osteoporosis were almost three times more likely, the occurrence of anemia was more than 40%, and chondrodystrophy almost 60% higher among patients with a TNF-α concentration greater than 100 pg / ml than in patients with TNF-α serum levels at concentrations below 100 pg/ml and the control group patients ($Z<Z0.05$). These data indicate that the general condition of the patients with high serum concentrations of TNF-α was more severe than the SG patients with normal or slightly increased content of this cytokine in the serum as well as in the control group patients. These data suggest

that MTLE associated with herpes neuroinfections, is not an exclusively neurological disease. This is a system process and sharp increases in the serum concentrations of TNF-α are a link between the cerebral pathological process and the development of changes in other organs and tissues spatially distant from the CNS.

On comparing the results of the MR tomograms of the brain, the signs of partial or focal lesions were revealed in the form of demyelination and necrosis with typical diffuse changes (in almost 70% of all the cases) and these were detected in the patients of the study group with high concentrations of TNF-α. At the same time, in the patients with normal serum levels of this cytokine, diffuse changes were detected, mainly associated with herpes neuroinfections, particularly, signs of brain atrophy, hippocampal sclerosis, selective atrophy of the temporal lobes, leptomeningeal fibrosis, hypertrophy of the choroid plexus of the ventricles and intracranial hypertension. In this regard, patients with high concentrations of TNF-α more often showed signs of focal neurological symptoms and deeper neurological deficits, and the degree of neurological deficit was closely related with the quality of life, the patient's ability to work and the need for home care.

No association was found to exist between the type of epileptic seizures and serum levels of TNF-α, although patients with high levels of this cytokine had more frequent polymorphic seizures, serial paroxysms and status epilepticus during the history of the disease prior to participation in the study, indicating an unfavorable course of the disease in these patients ($Z<Z0.05$).

Also, no definite association was found between the pattern of epileptiform activity on the EEG and serum concentration of TNF-α, although it should be noted that the abnormal EEG phenomena in the form of spikes, complexes of spike-waves and polyspike-waves in the interictal period were detected almost twice more often in the patients of the study group with serum cytokine concentrations above 100 pg/ml, than in patients with lower levels of TNF-α, or in patients of the control group ($Z<Z0.05$).

A higher degree of refractoriness to anticonvulsants was noted in patients with high serum concentrations of TNF-α. These patients changed a larger number of anticonvulsants during the history of the disease, took higher doses of anticonvulsants and often resorted to a combination therapy of antiepileptic drugs of different pharmacological groups. However, despite this, they had a significantly higher than the average frequency of epileptic paroxysms than patients with lower levels of this cytokine ($p<0.05$; $Z<Z0.05$) (Fig. 2). Perhaps this difference was influenced by the fact that the average duration of the epileptic syndrome before the onset of participation in the study was significantly greater in patients with high serum concentrations of TNF-α ($p<0.05$; $Z<Z0.05$) (Fig. 3). It is also possible to explain the antagonistic activity of TNF-α on the effects of the anticonvulsants, which produce a stabilizing effect on the membrane of neurons [54]. The same applies to the effectiveness of the antiviral therapy. Partial or complete remission of the seizures after treatment with acyclic guanosine analogs and immunotherapeutic interventions was rarely achieved in patients with signs of systemic inflammatory response ($p<0.05$; $Z<Z0.05$).

Discussion

Thus, the results obtained substantiate the possibility of estimating TNF-α as a biomarker of severity and prognosis in patients with herpes neuroinfections with epileptic syndrome. High serum concentration of this cytokine (above 100 pg/ml) is associated with more severe progression of MTLE and with the general condition of patients, and the poor prognosis of the disease. The inclusion of this objective laboratory test in the diagnostic algorithm to identify and monitor patients with virus-induced epileptic syndrome will certainly improve the quality of medical services by optimizing the diagnostic and therapeutic interventions. This is quite an informative and objective test, characterized by the relatively low cost and speeds of getting the results, to avoid some mistakes in evaluating the severity of the patient and prognostic aspects. The dynamic performance of this test (e.g. a once a month) can be very useful in monitoring patients with refractory convulsive syndrome associated with herpes neuroinfections. For the first time, TNF-α has been suggested as a biomarker for bacterial sepsis to evaluate the severity of SIRS and predict the further course of the pathological state. In addition, serum cytokine concentration above 1000 pg/ ml is still regarded as a prognostically unfavorable sign, associated with mortality [32]. Later, TNF-α was used to assess the inflammatory activity in rheumatoid arthritis, sarcoidosis and Crohn's disease, which was associated with the abnormal activity of the T-helper type 1 cells.

Recently, the TNF-α was used as a biomarker of the severity and prognosis in heart failure. It has been established that an increase in the serum concentrations of this cytokine is associated with a high risk of cardiovascular decompensation. Pentoxifylline (an inhibitor of the phosphodiesterase), capable of inhibiting the TNF-α production, possibly by the adenosine-dependent pathway [22], has demonstrated efficacy in the treatment of heart failure in the patients with high initial serum concentration of this cytokine in several smaller studies [47]. The first positive results on the effectiveness of anti-inflammatory therapy were obtained for Parkinson's disease (cyclosporine in an experimental model [54] and ibuprofen in patients with this disease [55]) which is characterized by its presence in the pathogenesis of cerebral inflammatory reaction.

Thus, our results open the way to optimize the treatment of the MTLE by overcoming the refractoriness to anticonvulsants in patients with verified herpes neuroinfections. Our data are consistent with the results of similar research works in other neurodegenerative diseases. The differences in the prediction, sensitivity to the antiviral and anti-convulsive drugs in patients with different levels of serum TNF-α justify its use as a criterion for the selection of treatment. On identifying the high content of tumor necrosis factor-alpha (greater than 100 pg/ml) more aggressive therapeutic strategy can be used for the inhibition of viral reproduction and relief of the epileptic syndrome; however in a lower concentration of this cytokine a mild anticonvulsant and antiviral treatment can be used. Patients with high serum concentration of TNF-α, which is associated with a high risk of the formation of additional lesions and focal

epileptiform activity, are recommended to undergo a more prolonged and careful monitoring with control brain MRI and EEG. This approach will allow, over time, to optimize the antiviral and anticonvulsant medication. Finally, for the patients with high serum concentrations of TNF-α with pronounced systemic effects and severe refractory epilepsy, a short-term specific anti-inflammatory therapy may be recommended with etanercept (a soluble neutralizing receptor for TNF-α) [56] and the monoclonal antibodies (infliximab [57], adalimumab [56], certolizumab [58]). This therapy is able to improve the patient's general condition and increase the sensitivity to the antiviral and anticonvulsant drugs, by rapidly blocking the negative effects of TNF-α. Some of these drugs have proved to be effective in the treatment of immune diseases such as rheumatoid arthritis, sepsis, Crohn's disease and sarcoidosis [57, 32]. At the same time, it must be considered that chronic treatment with these drugs of the monoclonal antibodies could be associated with the risk of deterioration of the patient condition by inducing secondary immunosuppression and facilitating viral reproduction [59]. In addition, there are reports on the effectiveness of the statins, antiatherosclerotic drugs with anti-inflammatory activity in arresting seizures in animal experiments [60]. In light of these studies, such drugs may be potentially useful in the treatment of epilepsy, particularly in older patients with vascular risk factors.

The results of our study can widen our current understanding of the pathophysiological aspects of TNF-α, which is a powerful pro-inflammatory cytokine that can mediate the inflammation in the area of the epileptic foci and exert direct and indirect proepileptogenic effects, as it is able to potentiate the reproduction of the herpes viruses and produce several adverse systemic effects in patients with MTLE associated with herpes neuroinfections.

Conclusions

In patients with MTLE associated with herpes neuroinfections significantly higher concentrations of TNF -α in serum are noted, when compared with patients without them. This fact implies the presence of a systemic inflammatory response that affects the severity of the epilepsy and the general condition of the patient. Therefore, MTLE with herpes neuroinfections should be considered not only as a neurological disease, but also as a systemic one.

The serum concentrations of TNF-α greater than 100 pg/ml are associated with more severe epileptic syndrome, a worse general condition of the patient, more pronounced changes in the MRI of the brain accompanied by profound neurological deficits, low sensitivity to antiviral drugs and anticonvulsants, as well as the poor prognosis of MTLE associated with herpes neuroinfections.

The serum concentrations of TNF-α can be used in the practice of epileptologists, neurologists, neurosurgeons, infectious disease specialists and clinical immunologists as an informative biomarker of the severity and prognosis in patients with MTLE. This becomes significant for timely selection of the best strategies for monitoring, treatment, and prevention of the disease.

However, it is necessary to conduct an independent study with greater statistical power to finally decide on a wide practical application of serum concentrations of TNF-α as a biomarker in MTLE associated with herpes neuroinfections.

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