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Role of IL-6 and neopterin in the pathogenesis of herpetic encephalitis

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Abstract:
Herpetic encephalitis (HSE) is one of the most severe infection of the central nervous system (CNS), connected with high mortality rate, even when appropriate therapy has been introduced. Better understanding of pathomechanisms responsible for neuronal injury during the course of the disease can be useful in the assessment of the risk of the occurrence of severe complications, as well as in potential introduction of additional therapeutic methods. The purpose of this study is to assess the correlation between concentration of neopterin and IL-6 in the CSF and serum, and the course of HSE. In this study, 36 patients with HSE were investigated, and the control group consisted of 32 patients in whom the infection of the CNS was excluded. We observed significantly higher concentration of neopterin and IL-6 in the CSF of patients with HSV as compared with the control group. Neopterin and IL-6 levels in the CSF correlated with the course of HSE. Higher values were connected with the risk of respiratory failure, development of permanent neurologic complications and patient death. Negative correlations between concentration of IL-6 and neopterin and patient condition assessed by Glasgow Coma Scale (GCS) were observed. Neopterin with high sensitivity and specificity allowed to predict the risk of death or severe neurological complications. Increased concentration of neopterin and IL-6 in the CSF and serum revealed reciprocal positive correlation. Assessment of the concentration of IL-6 and neopterin in the serum was not useful to predict the course of HSE.

Key words:
herpetic encephalitis, neopterin, IL-6, pathogenesis, prognosis, complications

Abbreviations: BBB – blood brain barrier, CSF – cerebrospinal fluid, CT – computed tomography, GCS – Glasgow Coma Scale, HSE – herpetic encephalitis, IL-6 – interleukin 6, MRI – magnetic resonance imaging, ROC – Receiver Operating Characteristic Curve

Introduction

Herpetic encephalitis (HSE) is the most common form of sporadic encephalitis among immunocompetent individuals. In most of the cases infection is due to the reactivation of the herpes simplex virus type 1 (HSV-1) from sensory ganglia, which then spreads by nerves to the central nervous system (CNS) causing changes in temporal and frontal lobes. In spite of antiviral therapy with acyclovir, HSE is connected with serious prognosis. As many as 30% of patients die or develop serious neurological complications during the course of the disease. According to some authors, if the treatment is delayed only 2.5% of patients who survived HSE will present with any abnormalities. In the remainder of patients memory deficits, dysphasia, behavior changes, and seizures will be observed [14, 20, 21]. These complications can significantly affect the patient's daily life, and usually the patients will not be

able to return to their previous professional activities. It should also be stressed that the introduction of steroids to the therapy is still controversial and requires further investigation [13, 16].

Understanding the pathogenesis of the injury of the CNS during the course of HSE and determination of factors connected with the risk of progression and prolonged course of this disease, will allow for easier identification of patients who need more intensive supervision and introduction of additional therapeutic methods.

Currently, few publications have connected the pathomechanism of the CNS injury in the course of HSE mainly with proinflammatory cytokines and chemokines, which also play a crucial role in the development of a defence mechanism against viral infection [3, 11]. Interlukin-6 (IL-6) is the cytokine that has mainly proinflammatory activity and plays an important role in inflammatory processes in the CNS. Current investigations indicate that it can contribute to an increase in permeability of the blood brain barrier (BBB) and stimulate proliferation of glial cells. Although high concentrations of IL-6 can have neurotoxic impact, there are also data, that suggest this cytokine may have a neuroprotective role [7–9, 21]. The concentration of neopterin, a marker of activation of cellular immunity, correlates with the intensity of oxidative stress during the course of inflammatory reaction, and is also associated with the neuronal injury during the course of HSE. Neopterin is mainly produced by monocyte/macrophages after stimulation with interferon-γ (IFN-γ). In the CNS, microglia cells are also the source of neopterin. Assessment of neopterin concentration allows to estimate the level of cellular immunity activation, as well as monitor and predict the progression of the disease [3–5].

The aim of the study was to assess the clinical significance and role of neopterin and IL-6 during the course of HSE and the correlation between the concentration of investigated markers in cerebrospinal fluid (CSF) and serum, and disease progression.

Materials and Methods

Sixty nine patients hospitalized during the last 10 years in the Intensive Care and Neuroinfection Unit of Infectious Diseases Department in Kraków were investigated: 36 patients with HSE and 33 (control group) admitted with the suspicion of meningoencephalitis, in whom on the basis of diagnostic procedures the neuroinfection was excluded. All patients underwent a lumbar puncture, and general examination of the resultant CSF was performed.

In all patients the concentration of neopterin and IL-6 in CSF and serum were measured on the first day of admission to the hospital. Material was stored at –70°C until the determination was performed. Quantitative measurement of neopterin and IL-6 was done with ELISA using the IBL IMMUNO-BIOLOGICAL and DIACLONE test in the Diagnostic Department of the University Hospital in Kraków.

Diagnosis of HSE was established on the basis of a clinical picture, CSF investigation which included polymerase chain reaction (PCR) and/or serologic test for HSV virus, as well as imaging studies, specifically computed tomography (CT) or magnetic resonance imaging (MRI) of the brain. The Glasgow coma scale (GCS) and neurologic examination were used to assess condition of the patients with HSE. CT scans revealed pathologic changes in 19 patients (53%), such as brain edema, hypodense lesion characteristic for HSE in frontal and temporal lobes (Tab. 1). In some patients MRI of the head also was performed.

Tab. 1. Characteristic of patients including assessment of changes on CT scan, number of points according to GCS, respiratory failure, neurologic complications and patients death

Sex	36 ♀ 33 ♂
Patients age	41.2 average 18 min., 73 max.
Presence of CNS infection	36 herpetic encephalitis (HSE) 33 control group
CT scan of the head	19 (53%) altogether 17 (47%) brain edema 9 (25%) changes in temporal lobe 4 (11%) changes in frontal lobe
GCS in HSE – number of scores	9 average 3 – min., 13 – max.
Respiratory failure which required mechanical ventilation	13 (36%)
Permanent neurological complications	6 (17%) altogether 2 (5.5%) vegetative state 4 (11%) severe mental retardation
Death during the course of HSE	4 (11%)

Patients with HSE were treated with acyclovir 10 mg/kg/d intravenously (*iv*) for 21 days. All patients with encephalitis, on the basis of providers' decision, received glicocorticosteroids (dexamethazone) *iv*.

Complications observed during the course of HSE included: respiratory failure with 13 (36%) patients requiring mechanical ventilation, death of 4 (11%), development of serious permanent neurological complications in 6 (17%) cases. Detailed patient characteristics are presented in Table 1.

In statistical analysis, the Mann-Whitney test was used to analyze differences in concentrations of IL-6 and neopterin between study groups. Correlation between neopterin and IL-6 concentrations in CSF and serum was assessed on the basis of Spearman's rank correlation coefficient. The same method was used to establish correlation between concentrations of investigated substances, patient's age, protein concentration in CSF, and the GCS score. In the group of patients with HSE, using the method of Receiver Operating Characteristic Curve (ROC), an attempt was made to determine a critical value of neopterin and IL-6 concentration in CSF and serum, which would predict, with highest reliability, the risk of occurrence of severe complications and death.

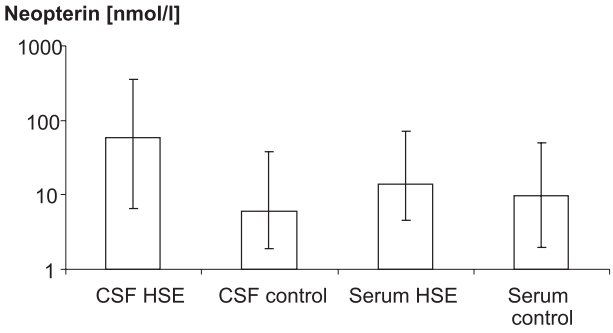

Fig. 1. Neopterin concentration in CSF and serum in patients with herpetic encephalitis and control group (control). Graph presents the arithmetic mean, minimal and maximal values. A statistically significant higher concentration of neopterin in CSF ($p < 0.01$) and serum ($p = 0.021$) was observed in the Mann-Whitney test in group with HSE compared with control

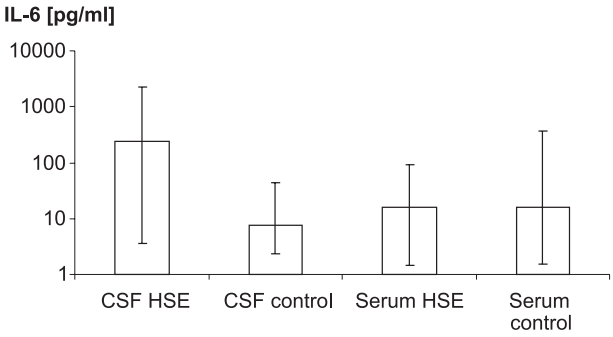

Fig. 2. IL-6 concentration in CSF and serum in patients with herpetic encephalitis and control group (control). Graph presents the arithmetic mean, minimal and maximal values. A statistically significant higher concentration of IL-6 in CSF ($p < 0.001$) and serum ($p = 0.001$) was observed in the Mann-Whitney test in group with HSE compared with control

Results

A statistically significant difference in the concentration of neopterin and IL-6 between both investigated groups was observed in the Mann-Whitney test. Higher concentration of neopterin ($p < 0.001$) and IL-6 ($p < 0.001$) in CSF and neopterin in serum ($p = 0.021$) was observed in patients with HSE as compared with the group without infection of the CNS (Figs. 1, 2), whereas IL-6 concentration in serum was higher in the control group ($p = 0.001$) (Fig. 2).

Among patients with HSE, Spearman's rank correlation coefficient for neopterin and IL-6 concentration in CSF and serum was $r_s = 0.274$ for $p < 0.023$, $r_s = 0.685$ for $p < 0.001$, $r_s = 0.436$ for $p < 0.001$, respectively. The coefficient for IL-6 in CSF and neopterin and IL-6 in serum was $r_s = 0.357$ for $p = 0.003$, $r_s = 0.384$ for $p = 0.001$. For neopterin in serum and IL-6 in serum r_s coefficient was 0.494 for $p < 0.001$ (Fig. 3).

Inversely proportional, statistically significant correlation ($p < 0.05$) between concentration of neopterin

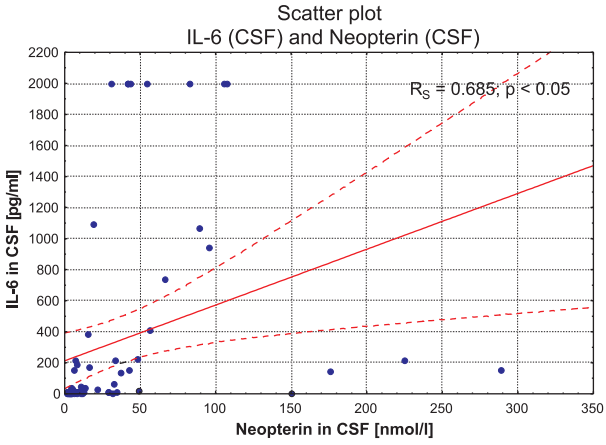

Fig. 3. Correlation of neopterin and IL-6 in CSF, Spearman's rank correlation coefficient 0.685 for $p < 0.001$

and IL-6 in CSF, and concentration of IL-6 in serum and GCS scores at the day of admission was observed. The correlation coefficient was r_s = –0.48, –0.61 and –0.36, respectively.

Patients who died or developed permanent neurological complications had a statistically significant increase of neopterin (p = 0.013) and IL-6 (p = 0.05) concentration in CSF as compared with the group who recovered without any sequelae. A similar relationship was noticed in the case of patients who had changes in the brain on CT (p = 0.012, p = 0.052) and who required mechanical ventilation (p < 0.001 for both determinations). In the case of neurologic complications and changes on CT, the difference was on the border of statistical significance (p ≤ 0.05). It was noticed that patients who required mechanical ventilations had statistically significant increase of IL-6 (p = 0.023) in CSF. Such a correlation was not observed for neopterin and IL-6 level determined in serum. Also there was no relationship between neopterin and IL-6 measured in CSF and serum, and protein level in CSF, or age and sex of patients.

Conducted analysis revealed a relationship between elevated concentrations of IL-6 and neopterin in CSF and the course of HSE. As a result of this finding, we used ROC curves to establish the level of IL-6 and neopterin above which the risk of death was increased. A critical point for neopterin, which provided a means to distinguish cases connected with the risk of death, was 96.1 nmol/l with sensitivity 75%, and specificity 90.3%. Also, concentration of IL-6 in CSF statistically significantly correlated with the risk of death. However, achieved results had a lower power of discrimination. The area under the ROC curve was greater for neopterin (AUC = 0.863). The highest prognostic value for the occurrence of neurologic complications according the ROC curves had neopterin. Diagnostic power of this test on the basis of AUC analysis was 0.828 and was statistically significant (p < 0.001). The critical point for neopterin, which allowed differentiation of cases connected with the risk of development of serious neurological complications, was 42.09 nmol/l and was characterized by 100% sensitivity and 62.1% specificity. Assessment of IL-6 concentration in CSF was also useful to predict the risk of the occurence of permanent neurological complications. The diagnostic value of IL-6 concentration was slightly lower than for neopterin (AUC = 0.756, p = 0.012). The optimal cut-off point for this measurement was 983.34 pg/ml and was connected with 66.7% sensitivity and 79.3% specificity. The discrimination power for measurement of neopterin and IL-6 in serum was 0.670 and 0.568, respectively, and in both cases there was no statistically significant correlation between investigated substances and the risk of complications.

Discussion

The patomechanism of CNS injury during the course of HSE has not yet been unambiguously elucidated. Neuronal destruction most likely results from both, direct cytophatic influence of the virus, as well as inflammatory reaction mediated by released components from dying cells. Until now, few investigations on patients with HSE, as well as on mouse model of the disease, revealed that cytokines and chemokines take part in development of defense mechanism against viral infection and injury of the brain tissue [1, 6, 11, 18].

The aim of the study was to assess the role of IL-6 and neopterin in the pathogenesis of the HSE.

A significant increase of neopterin and IL-6 concentration in CSF of patients with HSE was observed in comparison to the control group, which suggests their role in pathogenesis of CNS during the course of HSV infection. The correlation between the level of investigated substances, and the patient's condition at the admission was assessed on the basis of the GCS; changes on CT and MRI; necessity of mechanical ventilation; occurrence of permanent neurological complication or death also was observed. IL-6 and neopterin levels were higher in cases with severe course of HSE. This observation correlates with the Kamei et al. study which revealed increased concentration of IL-6 in CSF of all patients with HSE [9]. They also noticed that in the group of patients with poor prognosis maximal levels of IL-6 were significantly higher than in the control group [9]. This could indicate that prolonged activation of the immune system can play a crucial role in the development of severe complications [12, 18].

The result of other studies of viral encephalitis for etiologies other than HSV, showed the correlation between increased concentration of proinflammatory cytokines (TNF-α, IL-6, IL-8) in CSF and poor prog-

Tab. 2. Receiver Operating Characteristic Curve (ROC) and critical point for neopterin (nmol/l) and IL-6 (pg/ml) concentrations in CSF, which allow to distinguish cases connected with the risk of death and development of serious neurological complications. AUC = area under the ROC curve

Death	Critical point	Sensitivity	Specificity	AUC	p
Neopterin	> 96.1	75.0	90.3	0.863	< 0.001
IL-6	> 1092.81	75.0	83.9	0.794	0.025
Neurological complications	Critical point	Sensitivity	Specificity	AUC	p
Neopterin	> 42.09	100.0	62.1	0.828	< 0.001
IL-6	> 938.34	66.7	79.3	0.756	0.012

nosis [2, 8]. Undoubtedly interesting are reports about relapses of HSE, during the course of which high levels of cytokines in CSF were observed without the presence of genetic material of the virus [17]. This is not surprising because at this phase of disease, the virus can replicate only inside the brain parenchyma. Studies on a mouse model of HSE showed that immunological response against the HSV-1 virus infection plays a crucial role in the pathogenesis of brain injury and is directly connected with death of infected animals [11, 12, 19]. Dvorak et al. showed an increased expression of IL-6 mRNA in brain tissue of mice infected with HSV in the acute phase of the illness, as well as during the first three weeks after the infection [7]. Increased production of IL-6 by glial cell infected with HSV was observed [10, 12].

Only a few reports address the role of neopterin in the pathogenesis of HSE. Aurelius et al. found increased level of neopterin in CSF of patients with an activated immune system during the course of HSE [3]. Investigations of the role of neopterin during different CNS diseases in children also showed its increased concentration also in encephalitis [4]. Based on the conducted analysis we revealed that concentration of neopterin was not only significantly increased, but also correlated with severity of the disease, and a concentration above 96.1 nmol/l was connected with the risk of death. It can be explained by the increased production of neopterin, which leads to prolonged oxidative stress and enhanced cytotoxic effect of reactive oxygen species (ROS). The last published studies conducted on mouse model of HSE confirm the role of oxidative stress as the response to viral infection in neuron injury [15].

In this study, the results regarding the concentration of neopterin in serum were less explicit. The neopterin level in the serum, as well as in CSF, was elevated in the case of HSE, and IL-6 concentration in the serum was lower as compared with the control group. Higher levels of IL-6 in the serum of patients in the control group can be explained by the presence of infection localized outside the CNS. Patients from the control group, in spite of the fact that neuroinfection was excluded, had fever at the moment of admission, with different final diagnosis (data not presented). This observation indicates that neopterin level in the serum can be a more characteristic marker of HSE as compared with IL-6.

Assessment of neopterin and IL-6 in the serum was not useful to predict the course of HSE and the possibility of complications. A significant increase in the IL-6 and neopterin levels in CSF indicates that CNS cells are the main source of them during the course of encephalitis.

On the basis of observed correlation between neopterin and IL-6 concentration in CSF and the clinical course of HSE, an effort was made to establish the level, above which the risk of the development of neurological complication was increased, and which one is the better marker of the disease progression. We demonstrated that neopterin in CSF is more sensitive and specific marker of HSE progression than IL-6 (Tab. 2). However the sensitivity and specificity of this test seems to be inadequate for predicting the risk of death or occurrence of complications in clinical practice.

The conducted study revealed the role of inflammatory response in the brain damage during the course of HSE. HSV infection causes microglia activation and production of immune mediators, which are responsible for virus elimination, but also can lead to the CNS injury [6, 10, 20]. As it was mentioned above, neopterin production cause ongoing oxidative stress. Neopterin biosynthesis can be connected with

nitric oxide synthase (iNOS) activity, and accumulation of nitric oxide [5, 15]. All these processes lead to the neurodegeneration. Also concentration of IL-6 correlate with HSE severity. Although IL-6 has some anti-inflammatory activity, its high concentration can enhance apoptosis, BBB permeability, and stimulate glia proliferation [7, 10]. The results of the study clearly indicate the role of neopterin and IL-6 in the injury of CNS during the course of HSE. The levels above which the risk of severe complications increase can be useful to make decision about introduction of steroids to the therapy, which, according to recent publications, can be beneficial for the patients, and improve their prognosis [13, 16].

Conclusions

1. IL-6 and neopterin levels in CSF in patients with HSE are significantly higher than in patients without neuroinfection, which indicate their role in pathogenesis of the disease.
2. IL-6 and neopterin levels in CSF correlate with the course of HSE. High levels of IL-6 and neopterin in CSF are connected with the occurrence of permanent neurological complications and death. It seems that neopterin is a better prognostic marker, but its sensitivity and specificity does not allow to use it in clinical practice.
3. The assessment of IL-6 and neopterin concentrations in the serum is less useful to predict the course of HSE. However, an increase of IL-6 and neopterin serum levels correlate with their increase in CSF.

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