

COMPARATIVE ANALYSIS OF CLINICAL AND LABORATORY PARAMETERS BETWEEN VIRAL AND BACTERIAL NEUROINFECTIONS

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ABSTRACT

Neuroinfections are acute inflammatory diseases of the central and peripheral nervous system that can lead to serious consequences, and even death. Recently, viruses have played a leading role in the emergence of neuroinfections. Rapid identification of the etiological agents is an important prerequisite for proper therapy and a good outcome of the disease. The aim of this study is to determine the role of the cytokines IL-6 and IFN- γ in the cerebrospinal fluid and serum of patients with viral and bacterial neuroinfections in relation to their diagnosis and prognosis. Materials and methods: From 2012-2018, 91 patients were included, aged from 2 months to 82 years. They were divided into 3 groups: 57 with viral neuroinfections, 24 - with bacterial and 10 - control group with cerebral edema. Clinical, epidemiological, laboratory, microbiological, serological and molecular tests were performed in all patients, and in some of them imaging techniques (CT and MRI) had been performed. Cytokines IL-6 and IFN- γ in serum and cerebrospinal fluid were determined by immunological tests. Conclusion: Viral neuroinfections are more common than bacterial ones, they had a milder clinical course

and a more favorable outcome. Cytokine levels in the cerebrospinal fluid are a better indicator of inflammatory process in terms of severity than those in the serum. IL-6 levels in the cerebrospinal fluid of viral neuroinfections were higher than IFN- γ . A proportional relationship was established between leukocytes and IL-6 in the cerebrospinal fluid of patients with aseptic meningitis.

Keywords: neuroinfections, IL-6, IFN- γ , prognosis

INTRODUCTION

Neuroinfections are acute inflammatory diseases of the central and peripheral nervous system (CNS and PNS) that can be severe thus leading to serious consequences even death. They are caused by a wide group of etiological agents: bacteria, viruses, rickettsia, mycoplasmas, chlamydia, fungi, and parasites. Recently, viruses have played a leading role in the emergence of neuroinfections. Regardless of the etiological agent, neuroinfections are clinically similar. It is difficult to distinguish between them without specific microbiological, virological and serological tests.

The aim of the study was to determine the diagnostic and prognostic role of the cytokines Interleukin-6 (IL-6) and Interferon- γ (IFN- γ) in the cerebrospinal fluid (csf) and serum (ser) of patients with viral and bacterial neuroinfections.

Materials and methods: From 2012-2018 91 patients, 43 women and 48 men, aged from 2 months to 82 years (\bar{x} 19.8 \pm 23.4) were involved..

The diagnostic criteria for meningitis/encephalitis included clinical manifestations as well as laboratory changes in the csf of all patients. The clinical findings observed varied including:

- sudden onset
- headache
- fever
- nausea and/or vomiting
- neurological signs of meningeal irritation: neck stiffness, Kernig's sign, upper and lower Brudzinski's sign
- possible paralysis of the cranial and peripheral nerves
- pathological reflexes
- impaired consciousness

According to the relevant laboratory biochemical parameters in csf, the patients were divided into 3 groups:

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Group 1. Patients with aseptic (viral) neuroinfections.

This group consisted of 57 patients, from 11 months to 82 years (\bar{x} 15.74 \pm 19.32). The group was differentiated by the presence of aseptic meningitis, encephalitis or meningoencephalitis determined by:

- increase in albumin level usually below 1,0 g/L
- pleocytosis up to 1000.10⁶/L
- prevalence of lymphomononuclear cells
- slightly positive Pandy's and Nonne-Apelt's tests
- serological and/or molecular-based evidence of a virus as an etiological agent
- negative microbiological test for bacterial flora.

Group 2. Patients with purulent (bacterial) neuroinfections.

This group consisted of 24 patients aged between 1 month and 76 years (\bar{x} 17.07 \pm 11.04). Laboratory criteria included:

- albumin above 1.0 g/L
- increased cell count more than 1000.10⁶/L
- neutrophil proliferation
- strong positive tests of Pandy and Nonne-Apelt
- glucose level below 2,22 mmol/L
- positive microbiological and/or serological and molecular-based data of a bacteria agent

Group 3. Controls. The control group involved 10

patients aged from 1 to 56 years (\bar{x} 15.29 \pm 17.14). They were admitted based on clinical symptoms and history of cerebral edema. The following CSF parameters were within the reference values:

Clinical, epidemiological, laboratory, microbiological, serological and molecular based tests were performed in all patients. Computed tomography (CT) was used in all and magnetic resonance imaging (MRI) - only in selected patients. Concentrations of IFN- γ and IL-6 in csf and ser were examined in all patients. Cytokine levels were measured by enzyme linked immunosorbent assay (ELISA) using commercially available kits (Bender MedSystems GmbH (eBioscience), Vienna, Austria) according to the manufacturer's instructions. The sensitivity of the assay was set at 0.92 pg / ml for Il-6 and 0.99 pg / ml for IFN γ . The data was processed using the statistical program IBM SPSS Statistics v.19. All data was analyzed and included in the study after obtaining of informed consent from all participants.

Results: The neuroinfections in the studied group of patients were caused by arious etiological agents, as indicated in **Fig 1**.

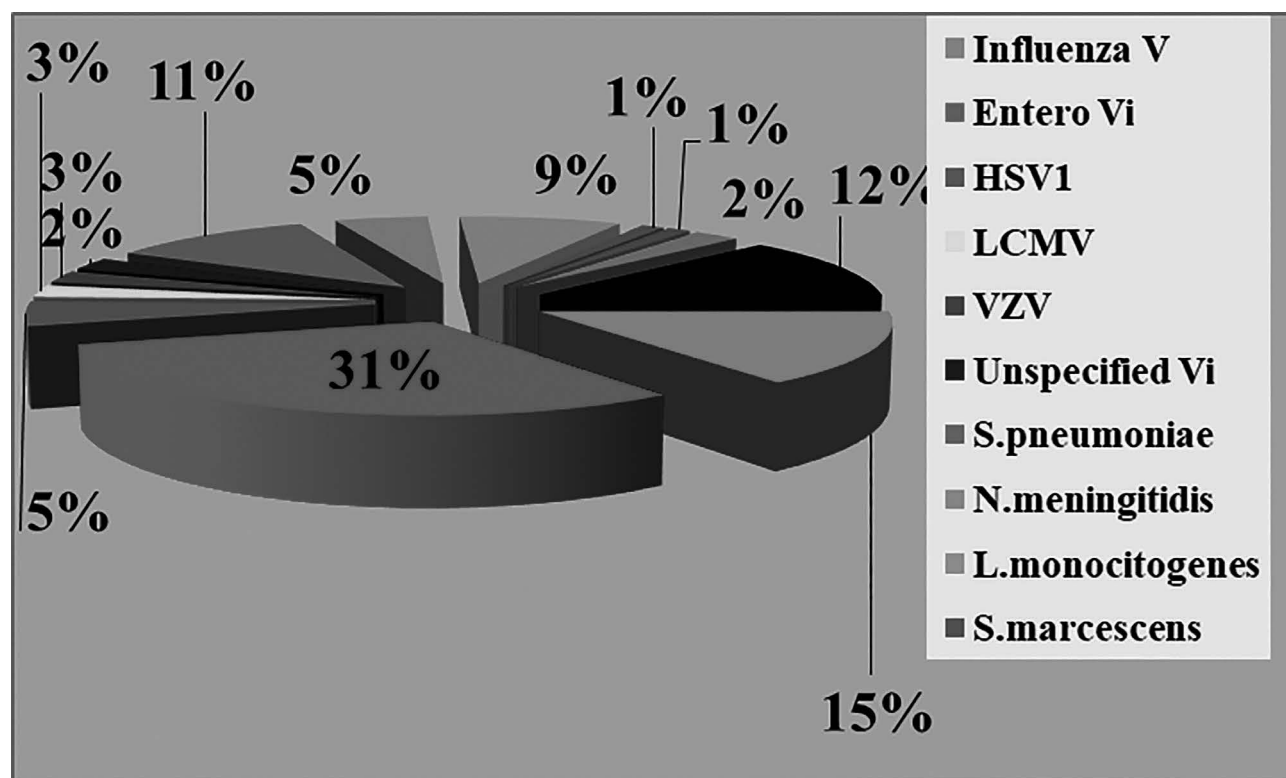


Figure 1. The etiological structure of neuroinfections - Influenza virus (IV), Enterovirus (EV), Hereps simplex virus 1 (HSV1), Lymphocytic Choriomeningitis virus (LCMV), Varicella-Zoster virus (VZV), Unspecified virus (UV), *Streptococcus pneumoniae* (*S. pneumoniae*), *Neisseria meningitides* (*N. meningitides*), *Listeria monocitogenes* (*L. monocitogenes*), *Serratia marcescens* (*S. marcescens*)

Group 1. The majority showed an acute onset of disease with headache, fever and vomiting. Fever was seen in all patients from 37.5 to 40.5° C (\bar{x} 38.4 ± 2.5) with a duration of 2 to 8 days (\bar{x} 3.32 ± 3.19). Headache was reported in 48 patients (84.21%). Vomiting was shown as a common sign in 46 (80.70%) patients with viral neuroinfections. Abdominal pain was observed in 37 (64.91%) patients and diarrhea was seen in 32 (56.14%). In most of these patients, EV was mostly found, followed by IV. Catarrhal manifestations such as , sore throat, runny nose and cough were more common in IV neuroinfections. There were 53 patients with severe asthenia upon hospitalization. Meningeal syndrome was found in 49 patients (78.76%).

Group2. Complaints also began acutely within 1–3 days with headache, vomiting, and temperature of 37.6 to 40.1° C (\bar{x} 38.8 ± 0.72). Neurological symptoms of meningeal syndrome were observed in all patients. Changes in consciousness were significantly more frequent as compared to the other two groups,. Rash was commonly found in meningococcal and pneumococcal meningitis.

Group3. These patients were in the control group diagnosed with cerebral edema. All had temperature in the range of 38.1 to 39.8° C, headache and vomiting, as well as partially manifested neurological signs of meningeal irritation syndrome. Due to a suspicion of neuroinfection, a lumbar puncture was done. The status of the patients was monitored during the hospital stay, as well as their laboratory parameters and this strictly excluded CNS inflammation.

Ten (17.55%) patients from the Group 1 were hospitalized within 3-7 days after the onset of symptoms. Dyspeptic manifestations such as abdominal pain and diarrhea were demonstrated in the three comparable groups, they all had similar frequencies. In viral infections of the CNS, they were observed mainly in EV ones. However, vomiting was not perceived as a manifestation of dyspeptic syndrome, but as a part of the neurological symptoms. In contrast to the classical literature nausea was a common symptom.

Meningeal symptoms were well expressed in

most patients from the three groups. Our study showed that the signs of meningeal irritation were more common in EV than in IV neuroinfections. The duration of meningeal syndrome was slightly longer in bacterial neuroinfections (\bar{x} 6.61 ± 2.21) as compared to viral (\bar{x} 4.54 ± 3.82). In the control group, meningeal irritation lasted an average of 3.02 ± 0.11 which was not significantly different. Seizures were initially observed before or shortly after hospitalization in groups 1 and 2. In viral neuroinfections they are most common in IV and HSV, while in bacterial neuroinfections - in *S. pneumoniae* and *N. meningitidis*, respectively. Quantitative disorders of consciousness were common. A statistically significant difference was found between the three groups ($p < 0.001$ for all comparisons). Cranial nerve palsy was observed with comparable frequency in groups 1 and 2, while peripheral nerve palsy was more common in viral than in bacterial neuroinfections - ($p < 0.01$).

Almost all patients included in the study were admitted within 3 days after the onset of the disease. The time of hospitalization was important for the length of the hospital stay, this was done so as to minimize complications, reduce adverse reactions and eventually receive a positive outcome. The average length of hospital stay in bacterial neuroinfections was the longest: from 2 to 31 days (\bar{x} 14.21 ± 9.21), followed by viral infections: from 1 to 22 days (\bar{x} 9.12 ± 3.12), and the control group: from 3 to 5 days (4.11 ± 2.64). No statistically significant differences were detected between the groups for this parameter. Laboratory blood analysis showed leukocytosis with predominance of neutrophils and increased CRP in bacterial neuroinfections. Elevated urea, creatinine, ALAT and ASAT were found in most patients with neuroinfections but they were more common in those of bacterial origin. Statistically significant differences between the studied laboratory parameters were detected only vs. group 3: $p < 0.01$ for both comparisons. Laboratory characteristics of cerebrospinal fluid showed inflammatory changes corresponding to a bacterial or aseptic inflammatory process. There were no pathological abnormalities in the control group. (Table. 1.)

Table 1. Indicators of CSF at the initial lumbar puncture of the three patients' groups

Groups of patients Indicators / \bar{x} /	Group 1 (n=57)	Group 2 (n=24)	Group 3 (n=10)	P
Leuc.10 ⁶ /L	77,60±27,21	1112,87±382,22	2,43±2,11	1v2=0,003 1v3=0,43 2v3=0,000
Protein g/L	0,75±0,47	1,96±1,33	0,27±0,13	1v2=0,21 1v3=0,34 2v3=0,04
Glucosa mmol/L	4,15±2,98	2,04±0,23	3,15±1,19	1v2=0,003 1v3=0,43 2v3=0,000
Sed.(Ly%)	68,12±28,10	35,14±11,09	/	1v2=0,12

The results of the changes in IFN- γ and IL-6 in CSF and serum in the three groups of patients were presented in **Table 2**.

Table 2. Mean values of IFN- γ and IL-6 (pg/mL) in cerebrospinal fluid and serum in the groups of viral, bacterial neuroinfections and controls.

Groups of patients / Cytokines(pg/mL)	Group 1 (n=57)	Group 2 (n=24)	Group 3 (n=10)	P value
IFN- γ csf	2,15±7,97	96,70±235,29	2,20±1,04	*1vs2=0,000 *2vs3=0,000 1vs3=0,161
IFN- γ ser	0,22±0,62	0,45±1,00	0,0006±0,006	*1vs2=0,004 *1vs3=0,000 *2vs3=0,000
IL-6 csf	42,85±113,56	358,30±221,68	2,91±1,25	*1vs2=0,003 *2vs3=0,000 1vs3=0,231
IL-6 ser	6,09±12,34	30,61±28,54	4,39±12,02	*1vs2=0,000 *2vs3=0,000 1vs3=0,181

Levels of IL-6 and IFN- γ (pg/mL) in both cerebrospinal fluid and serum had been shown to be significantly higher in bacterial neuroinfections than in viral ones. Imaging methods of diagnosis: CT was done for all patients and MRI in 28. The most common finding was cerebral edema. The MRI in five patients with HSV1 neuroinfection demonstrated the classic picture of multistage hemorrhagic-necrotic changes in the brain parenchyma in the frontotemporal regions. The outcome of neuroinfections in our patients was often favorable. Fatal outcome was observed in 8 patients with viral (14.03%) and 8 (33.33%) with bacterial neuroinfection, no statistically significant difference was found. Residual manifestations - palsy of cranial nerves, most often VI and VII, was observed

in 3 patients (5.26%) with viral (HSV1, LCMV) and in 5 (20.83%) with bacterial neuroinfection (*S. pneumoniae*, *N. meningitidis*), $p = 0.004$.

DISCUSSION

Cytokines are known to regulate the intensity and duration of immune response by stimulating or inhibiting the activity, proliferation and / or differentiation of various cells and controlling the secretion of other cytokines and antibodies. Recently, much attention has been paid to the role of cytokines in the regulation of inflammation and host responses to CNS infection [1]. According to literature data, the release of some cytokines, such as interleukin-1 (IL-1), interleukin-8 (IL-8), tumor necrosis factor- α

(TNF- α) and interferon-gamma (IFN- γ), could be responsible for meningeal inflammatory infiltration in purulent and aseptic meningitis and may correlate with the outcome of the disease [2,3,4,5,6].

IL-6 elicits an inflammatory response during the acute phase, which manifests itself with fever and leukocytosis. It also contributes to the transition from acute to chronic inflammation [3]. The etiological diagnosis of bacterial neuroinfections is confirmed by visualization of the etiological agent on a direct Gram-stained preparation and / or by culturing the cerebrospinal fluid on a selective culture medium. The limited number of pathogenic species that could be detected in the laboratory, was most probably associated with the widespread use of pre-hospital antibiotics, which ultimately reduces the effectiveness of etiological diagnosis [7]. In viral infections of the CNS, the detection of genetic material of the causative agent in the cerebrospinal fluid by polymerase chain reaction (PCR) is the gold standard for diagnosis. This otherwise avant-garde method was not always successful, as there might be insufficient viral presence in the biological fluids during the study. While affordable, easy-to-use and inexpensive methods for diagnosis of bacterial neuroinfections have been developed, this is more difficult in viral infections due to their great variety [8]. Bacterial meningitis was associated with the activation of the inflammatory cascade and the production of pro- and anti-inflammatory cytokines [3]. Using the levels of three cytokines, TNF α , IL-6 and IL-8 in the cerebrospinal fluid, some authors distinguish between bacterial and viral meningitis in children with 100% specificity and sensitivity. In our study, a significant difference was found between viral and bacterial neuroinfections with respect to the IL-6 and IFN- γ (pg /mL) in serum and cerebrospinal fluid. Other authors have reported similar observations [3]. IL-6 levels in the cerebrospinal fluid of patients in group 2 were much higher than those cited by Prasad, R et al. [3].

According to Ichiyama T, elevated concentrations of csf pro-inflammatory cytokines such as TNF- α , IL-1 β and IL-6 - showed evidence of acute encephalitis / encephalopathy [9].

In our study, the cytokine profile was examined in bacterial and viral neuroinfections in comparison to healthy controls. In the group of viral neuroinfections, the highest IL-6 values were found in the five patients

with herpetic meningoencephalitis. A study of csf cytokines (IL-1 β , IL-2, IL-6, IL-10, TNF α , and IFN- γ) in two cases of nonherpetic limbic encephalitis by Takahashi T et al, found elevated values of IL-6 only [10]. IL-6 elicits acute inflammatory response, followed by fever and leukocytosis. It also contributes to the transition from acute to chronic inflammation [6]. In our patients we found a directly proportional relationship between the level of IL-6 and the levels of leukocytosis, pleocytosis and CRP.

The sensitivity of csf IL-6 as a diagnostic marker in bacterial meningitis was significantly higher as compared to conventional physicochemical parameters such as leukocytes and protein. When the level of IL-6 in the cerebrospinal fluid exceeded 38.2 pg/mL, its diagnostic sensitivity as a biomarker was 100.0%, as compared to 70.0% for the extent of pleocytosis and 65.1% for proteinorachia [11]. In our study, the level of IL-6 in the cerebrospinal fluid of patients with bacterial CNS infections averaged to 358.30 \pm 221.68 pg/ml. In adult patients with bacterial meningitis, IL-6 levels were higher than in children, ($p < 0.001$). This was found by other authors and was explained by the maturity of the immune system of the adult organism [11].

Cytokines at the site of inflammation were a better indicator of the clinical severity of the disease than their serum levels. According to experimental results, the csf / ser IL-6 ratio had a higher diagnostic efficacy than the independent detection of IL-6 in csf [4].

Takahashi W et al. conducted a study in 70 patients, 13 of whom had bacterial meningitis, 21 with aseptic inflammation of the central nervous system and 36 with sepsis. The level of IL-6 in the cerebrospinal fluid was significantly higher in the group with bacterial meningitis as compared to the other 2 groups ($p < .0001$) [11]= This finding was confirmed in our study. We found a strong relationship between serum and cerebrospinal fluid cytokine levels and the etiological agent, which had the value of a diagnostic marker. The higher values of IL-6 and IFN- γ in both biological fluids, the more certain it were that there is a bacterial causative agent.

Mukai AO et al.confirmed this dependence and, like us, did not find a link between cytokine levels and subsequent complications of neuroinfection. It was concluded that cytokines were a good marker for distinguishing bacterial from aseptic meningitis, as well as an index for the intensity of inflammation

[12]. According to Ogha S et al. the levels of IFN- γ concentration was the highest in the cerebrospinal fluid of patients with aseptic meningitis [13]. Our results do not confirm this. In our study IFN- γ showed a positive correlation with disease severity, as found by other authors. [14].

CONCLUSION

Our study of neuroinfections conducted in 91 patients (57 with viral, 24 with bacterial origin and 10 as a control group with meningism), showed that:

1. Viral neuroinfections were more common than bacterial ones and with rare exceptions, had a milder clinical course and a more favorable outcome. Vaccine prophylaxis reduced the chances of viral neuroinfections in mumps, measles, rubella, chickenpox, poliovirus, influenza, rabies.
2. Cytokines in the cerebrospinal fluid were a better indicator of the clinical severity of the inflammatory process of the CNS than their serum levels.
3. In viral neuroinfections, cerebrospinal fluid IL-6 levels increased more than IFN- γ .
4. In aseptic meningitis, we found a proportional relationship between the level of IL-6 and the amount of pleocytosis

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