Virginia encephalitis accounts for 40-70% of all cases worldwide, central nervous system infections pose a diagnostic challenge because clinical manifestations are not typically pathognomonic for specific pathogens, and a wide range of agents can be causative.

**Materials and methods:** Within the framework of the study, two groups of 90 people in each were formed from the patients with neuroinfections admitted to our Center. Intrathecal synthesis (ITS) of total (unspecific) IgG in members of one of group (group of compare) was determined. Brain synthesis of specific antibodies (Ab) to some neurotropic pathogens (herpes simplex virus 1/2, cytomegalovirus, Epstein-Barr virus, varicella zoster virus, rubella virus, Borrelia) was studied in the second group of patients (group of interest). There were no statistically significant differences between groups by gender and age. Encephalitis and encephalomyelitis prevailed among patients of both groups.

**Results:** ITS of total IgG was established in 30 (33.3 ± 6.1 %) patients of the first group with IgG index more than 0.6 indicating on inflammatory process in CNS and no marked changes of CSF. ITS of specific Ab was determined in 23 of 90 (25.6 ± 4.6 %) patients included into group of interest. In more than half of cases Ab to several infectious agents were detected simultaneously. ITS of various specificity, in particular, to measles and rubella viruses, and VZV, known as MRZ-reaction, is characteristic of some autoimmune lesions of CNS, multiple sclerosis first of all. In fact, further research of 5 patients with MRZ-reaction confirmed their autoimmune failure of CNS. Detection of ITS in the CSF samples didn’t depend on concentration of specific Ab in serum and CSF and wasn’t followed by HEB dysfunctions which were observed with the same frequency in patients with or without ITS (13.0 % and 13.6 % respectively).

**Conclusion:** Specific Ab synthesis to several neurotropic pathogens in the CSF of significant part of examined patients was established. Thus, diagnostic value of ITS of specific immunoglobulins seems to be limited to cases in which autoimmune damage of the CNS is suspected.

**KEY WORDS:** neuroinfections, intrathecal synthesis, specific antibodies

**INTRODUCTION**

Central nervous system (CNS) infections pose a diagnostic challenge because clinical manifestations are not typically pathognomonic for specific pathogens, and a wide range of agents can be causative. Smear staining and culture growth have of limited value, particularly for viral infections. Meanwhile, viral encephalitis accounts for 40-70% of all cases worldwide [1-5].

Recently diagnosis of CNS viral disease was mainly based on serologic investigations, including detection of immunoglobulin (IgG) intrathecal synthesis and specific antibodies index. It is known that specific IgG circulated in the blood can be sometimes detected in CSF, but in smaller titeres. Therefore, persistence of the antibodies in CSF reflects rather the general immune response, not allowing to judge the presence and activity of the infectious process in the CNS. 'Early antibodies', immunoglobulin M or A (IgM/IgA), are not adequate indicator of acute brain infection also. In addition, IgA easily overcomes the blood-brain barrier (BBB).

Molecular methods nowadays have become the gold standard for the diagnosis of neuroinfections, however, cases of negative PCR are not uncommon, even when encephalitis was confirmed by clinical and visual signs. The reason of that may be, for example, short time (less than four days) after disease onset. Besides, viral propagation at lapse occurs in fact in brain parenchyma cells and only a few viral particles may release in the liquor [6-8]. In this case, the detection of ITS may be useful in making the correct diagnosis. The immunoglobulin synthesis in brain is provided by memory B lymphocytes which immigrate into the brain after the primary infection and settle in the brain tissue. The immigrated B-lymphocytes are antigen primed, affinity matured and isotype specific. They synthesize immunoglobulins locally in the perivascular lymphocyte cuffs [9] and their pattern is locally varying but rather stable over the initial diagnostic phase of the diseases [10].

During flair ups priming B cells multiply, forming small (up to 1 mm in diameter) aggregates in different brain compartments [11]. B cell aggregates appear already a few
days after disease onset and increasingly display the phenotype of tertiary lymphoid organs (TLOs). Initially it was assumed that ITS is a sign of a specific immune response, but further research has shown that ITS is determined not only in the case of an acute infectious process in the CNS, when the pathogen is in the brain parenchyma and the immune response effectors migrate into the brain tissue, causing damage (encephalitis, myelitis, meningoencephalitis). The antigen-independent activation of B cells with T-lymphocytes and Toll-receptors on B-lymphocytes is also possible. As a result, a polyspecific immune response develops from ITS of varying specificity at the same time. In other words, ITS is not necessarily an indicator of an acute infectious process, but may be a part of the polyspecific immune response, which is due to antigen-independent B cells activation [12]. Below we present the data allowing to estimate the diagnostic value of intrathecal antibody synthesis in the patients with several neuroinfections.

THE AIM
To study frequencies of intrathecal synthesis of specific antibodies in patients with inflammatory disorders of the CNS.

MATERIALS AND METHODS
This prospective study to estimate the role of intrathecal specific Ab synthesis in making correct diagnosis, took place from January 2016 until the end of December 2016 at The Center of Infectious Disorders of the Nervous System (Kyiv, Ukraine). CIDNS is an adult tertiary referral, infectious diseases hospital covering all country territory. It has 20 beds with approximately 200-250 admissions annually. Patients were enrolled in the study if they have clinical evidence of a CNS infection: fever >38°C, or febrile episode reported within the previous month; CSF abnormalities (>four white blood cells per mm3 or CSF proteins >0.4 g/L); at least one of the neurological signs (confusion, altered mental status, seizures, focal deficiency). Exclusion criteria included noninfectious CNS diseases (cerebral tumor, cerebral abscess, and neurosurgery within the previous two-four months), and meningitis without clinical manifestation of brain involvement. Patients below 18 years were not included in the study, since children with CNS infections are managed in other specialized centers. Two groups were formed from our patients: first one included 90 patients with 30 (33.3 %) males and 60 (66.6 %) females from 21 to 70 years old age (average 38.9 ± 9.8). Intrathecal synthesis of total (unspecific) IgG was determined in members of the group (group of compare). Brain synthesis of specific antibodies to some neurotropic pathogens (herpes simplex virus 1/2, cytomegalovirus, Epstein-Barr virus, varicella zoster virus, rubella virus, Borrelia) was studied in patients of the second group (group of interest). There were no statistically significant differences between groups by gender and age. Encephalitis and encephalomyelitis prevailed among patients of both groups.

Quantification of total IgG in serum (S) and CSF was carried out by immunoturbidimetry method on automatic biochemical analyzer Cobas 311 (Roche, Swiss). Intervals between blood and CSF sampling did not exceed one hour. IgG index (I\textsubscript{igG}) was calculated by formula: I\textsubscript{igG} = \frac{\text{tot. IgG}_{\text{CSF}} \times \text{alb}_{\text{CSF}}}{\text{tot. IgG}_{\text{serum}} \times \text{alb}_{\text{serum}}}

Quantitative study of specific Ab in serum and CSF was provided by standard IFA method with Euroimmun ELISA-test systems to Herpes simplex virus type 1/2 (Ab HSV 1/2), cytomegalovirus (Ab CMV), Epstein-Bark Virus (Ab EBV), Varicella zoster virus (Ab VZV), measles virus (Ab MSLs), Rubella virus (Ab Rub), Borrelia (Ab Bor) [13,14]. The level of ITS was calculated accordingly to H. Reiber [12].

Permeability of BBB and blood-lymphatic barrier was evaluated upon ratio of albumin concentration in serum and CSF (albumin coefficient, Q\textsubscript{alb}), taking into account age ratios [15]. The formula used in this study to compare levels of specific antibodies in the CSF and in serum sampled at the same time was that developed by Tibbling and Link [16]. This formula takes into account the level of specific IgG and albumin in the CSF and comparatively in serum, to verify that the IgG measured are only of intrathecal origin.

If the index obtained by the following formula: IgG index = \frac{\text{IgG}_{\text{CSF}} / \text{Albumin}_{\text{CSF}}}{\text{IgG}_{\text{serum}} / \text{Albumin}_{\text{serum}}} is over 0.7, it means that specific IgG originate in CNS. If the result is less than 0.7, the IgG measured in the CSF crossed from the blood [12].

The degree of BBB dysfunction was determined on the basis of Schliep and Felgenhauer criteria [17].

RESULTS AND DISCUSSION
The prevalence of specific antibodies to some neurotropic pathogens in patients of studied group was very high and ranged from 100% (Ab-EBV) to 25.6% (Ab-Bor). Presence of antibodies in the blood was accompanied by their appearance in the CSF, which is obviously explained by their diffusion and penetration through the BB barrier (fig. 1). It should be noted that specific antibodies were usually found in the same patient simultaneously in the blood and CSF. The only difference between the frequency of Ab in serum and CSF related of measles virus (88,9 ± 3,3% in sera and 73,3 ± 4,7% in CSF, p <0,05). The prevalence of antibodies to herpesviruses (HSV, CMV, EBV, VZV) in the sera and CSF in the group of interest were very close. This fact may be explained by a lower concentration of anamnestic post-mortem or vaccine-derived antibodies against measles and rubella, in contrast to herpesviruses, which periodically stimulate immune response during relapse. The relatively low rate of detection of Ab-Bor is resulted the relatively low incidence of the infection in this group. As was to be expected, the prevalence of antibodies to herpesviruses and measles virus in first group were close to the second one, while Ab to borrelia were not found.

As can be seen from the data presented, Ab to several pathogens including various herpesviruses was established in both the blood and CSF of virtually all patients examined. Thus, serology makes it impossible to clearly differentiate the etiological factor of the disease. Moreover, even
PCR cannot always help in solving problem: CSF samples obtained from almost 90% of patients at admission were negative for all pathogens of interest.

In 30 (33.3 ± 6.1%) patients of the first group, ITS of total IgG was identified with an index higher than 0.6, which is evidence of the inflammatory process in the CNS. The average age of patients with total IgG ITS did not differ from the average age of patients in the group and was 38.1 ± 9.9 years. At the same time, females prevailed (22 out of 30 - 73.3%) among patients with ITS IgG. It should be emphasized that in most patients with ITS IgG, there were no pronounced changes in CSF indices: the cytosis was less than 10 cells, the total protein was within the normal range. Most of them did not reveal dysfunction of the BBB / HLB.

ITS of specific antibodies, in the second group, was detected in 23 of 90 (25.6 ± 4.6%) patients examined. Ab to one pathogen were revealed in 11 (47.8%) patients, one patient (4.4%) has ITS to two, and 11 (47.8%) - to three and more pathogens. The latter to be considered as a polyclonal immune response of unclear origin. In one of the patient with a multiple sclerosis we found ITS against all of the pathogens examined, that confirmed autoimmune nature of the disorder. We compared the presence of ITS antibodies with the frequency of detection of antibodies in CSF (fig. 2). Antibodies to the herpes simplex virus and Epstein-Barr in CSF were present in all examined patients, but the frequency of intrathecal synthesis of antibodies to these pathogens when they were present in CSF was relatively low (30.4% and 34.8%, respectively). When antibodies to the measles virus were detected in the CSF, in most cases ITS antibodies to this virus were detected. At the same time, antibodies to CMV in CSF were present in 87% of those examined, and ITC to this virus was found only in 25% of patients in whom antibodies were detected in CSF. It should be noted that in 75% of patients in whom antibodies to borrelia were detected in CSF, their intrathecal synthesis was established.

These data clearly demonstrate that the detection of specific antibodies in the CSF cannot be considered as a tool useful in making correct diagnosis of CNS damage. At the same time, the diagnostic value of the method for determining ITS antibodies is also shown.

When ITS to only one of the pathogens is detected, the etiology of the CNS lesion becomes more apparent. It
was found, for example that ITS to borrelia is a specific test, confirming neuroborreliosis. According to our data, all patients with the ITS to borrelia had no intrathecal production of antibodies to other pathogens. At the same time, detection of antibodies to borrelia in the serum is not always sufficient for establishing the diagnosis of neuroborreliosis, since borreliosis is an endemic infection in some regions of Ukraine, therefore, the number of virus carriers, and recovered is quite high.

In our study, there were clinical cases where ITS assay results pointed on activation of the infectious. For example, intrathecal synthesis of Abs to VZV accompanied by highly positive PCR allowed to confirm the activation of VZV-infection in the patient. In two cases, the detection of ITS to borrelia allowed to confirm the clinical diagnosis of neuroborreliosis.

However, as noted above, in more than half of the patients ITS against two or three pathogens was detected. In some cases, ITS only to the measles virus was found. We believed the autoimmune conflict in the CNS developed in these cases. A number of studies from other authors have shown that ITS of varying specificity, in particular to measles, rubella, VZV ("MRZ-reaction") characteristic for a number of autoimmune lesions of the CNS, in particular for multiple sclerosis (MS) [18]. In MS, the cause of ITS is the formation of ectopic lymphoid tissue in the brain parenchyma and its subsequent activation by immunopathological mechanisms at which differentiation of the memory B-lymphocytes is stimulated in the absence of antigens, due to the so-called unspecific activation. In this case, the immune response can reflect the individual history of previous infections and immunizations and depends on the regional prevalence of certain infections [10]. In our study “MRZ-reaction” was found in two patients. In one case, ITS was detected for measles and VZV, in other one, to measles and rubella viruses. Subsequently, oligoclonal antibodies, which are characteristic of MS were detected in one of them.

A separate discussion deserves the role of the Epstein-Barr virus in the CNS pathology. There is evidence in the literature that Ab-EBV is often found both in the serum and CSF of patients with MS [18]. According to the authors, the Epstein-Barr virus acts as a trigger signal, getting into the central nervous system and persisting in glial cells long before intrathecal inflammation and the formation of the TLO [11]. Other authors believe that the activation of naive B-cells penetrating into the central nervous system is due mainly to primary acute EBV infection [19]. Because at the time of acute infection, the production of specific antibodies has not yet started, this hypothesis can explain the high (almost 100%) incidence of Ab-EBV in patients with MS and the low frequency of ITS Ab-EBV. The data we obtained also indicate 100% of the detection rate of antibodies in CSF to EBV in the patients examined, with the frequency of ITS being relatively low - 34.8% (Fig. 2). It is possible that EBV was a trigger for chronic CNS lesions, which developed on the underlying immunopathological process.

It should be noted that there were no significant violations of the functions of the GEB / HLB in the examined patients. The minor violations, according to the Schliepand-Felgenhauer criteria, were established only in 13.6% of patients without ITS and 13.0% with intrathecal antibody synthesis. That is, relatively high concentrations of specific antibodies in CSF were not associated with increased permeability of GEB / HLB, but due to their direct production in brain tissues.

The conducted research has outlined the possible directions of using the ITS method for etiological and differential diagnostics of infectious, inflammatory, demyelinating and vascular diseases of the central nervous system.

**CONCLUSIONS**

1. Intrathecal synthesis of specific immunoglobulins was established in 23 out of 90 (25.6 ± 4.6%) patients with CNS lesions. Ab to several neurotropic agents were simultaneously present in 52.2% of cases.

2. Detection of ITS did not depend on the concentration of specific antibodies both in the serum and CSF and was not accompanied by a violation of the GEB / HLB function. The incidence of GEB / HLB dysfunction in patients with, and without ITS was found to be the same (13.6% and 13.0% respectively).

3. In the absence of ITS, the presence of specific Abs in CSF depended on their concentration in the blood and the permeability of GEB / HLB.

4. Although ITS assay has significant limitations on specificity, it can be useful especially at the onset of the disease and a negative PCR result.

**REFERENCES**


DIAGNOSTIC VALUE OF SPECIFIC ANTIBODY SYNTHESIS IN BRAIN OF PATIENTS WITH NEUROINFECTIONS


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