Antibody indices – a growing analysis in the diagnosis of CNS diseases

Identification of the causative agent of infectious CNS diseases is essential for providing appropriate treatment.

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The antibody index (AI) is a measure of local antibody synthesis in the central nervous system (CNS) and represents an important and growing tool in neurological diagnostics. Determination of the AI supports diagnosis of acute CNS infections and some non-infectious inflammatory diseases such as multiple sclerosis (MS). The analysis can be performed easily in any routine laboratory using standardised assays for measuring antibodies in cerebrospinal fluid (CSF) supported by specialised software for calculating the AI.

CNS infections

Acute infections of the CNS have high morbidity and mortality and often present as medical emergencies. They can be caused by viruses, bacteria, protozoa or fungi. Manifestations include inflammation of the protective membranes surrounding the brain and spinal cord (meningitis), inflammation of the brain tissue (encephalitis), or a combination of both (meningoencephalitis). Symptoms can be unspecific, for example, fever, headache and altered mental state. There is potential for seizures, paralysis and coma.

Identification of the causative agent of infectious CNS diseases is essential for providing appropriate treatment. Diagnosis typically involves a combination of physical examination, blood tests, imaging studies and analysis of cerebrospinal fluid (CSF) obtained by lumbar puncture (spinal tap)[1].

CSF analysis

Detection of specific antibodies in CSF supports the diagnosis of CNS infections. It is especially helpful in situations where direct methods such as PCR have a low positivity rate, for example, due to rapid pathogen clearance or in chronic stages of infection. The analysis is recommended by the German Society of Neurology (DGN) as part of the diagnostic work-up for suspected CNS infections. It is used alongside other CSF laboratory tests such as cell count, total protein, lactate, oligoclonal bands, cytology and direct pathogen detection.

In the DGN diagnostic guidelines for Lyme neuroborreliosis and

neurosyphilis, in particular, the detection of intrathecal antibodies against the respective causative organism (*Borrelia* or *Treponema pallidum*) serves as a main criterion for confirming a diagnosis[2, 3].

Multiple sclerosis

Some non-infectious inflammatory conditions such as MS trigger a polyspecific humoral immune response. In these cases, antibodies against multiple non-causative pathogens may be detected in the CSF. A reaction against measles virus, rubella virus and/or varicella zoster virus (MRZ) is a specific indicator of MS and can help to distinguish MS from clinically similar diseases. MRZ testing in patients with suspected MS is recommended by the German Society for Cerebrospinal Fluid Diagnostics and Clinical Neurochemistry in its current guidelines[4].

AI determination

In CSF antibody analysis, it is important to distinguish antibodies that are produced in the CSF from antibodies that have diffused from the blood into the CSF across the blood-brain barrier (figure 1). The AI takes the barrier function into account by setting the concentration of specific antibodies in CSF and serum in relation to total immunoglobulin.

The AI is calculated using the following formula: the quotient of pathogen-specific antibodies in CSF to serum (Q_{spec}) divided by the quotient of total IgG in CSF to serum (Q_{IgG}) (figure 2). An AI of more than 1.5 indicates pathogen-specific antibody synthesis in the CSF.

If total IgG in CSF is elevated, as occurs with polyclonal antibody stimulation in MS, the quotient of albumin in CSF to serum is additionally measured as a counterbalance. This is used to derive the limes quotient, which is used instead of the total IgG quotient in the calculation of the AI[5].

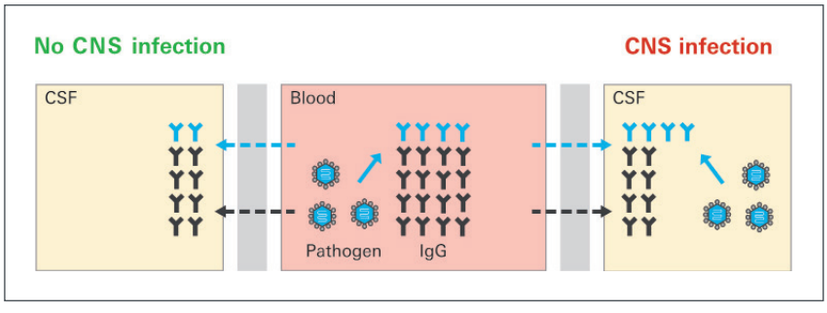
ELISAs for CSF/serum pairs

Specific antibodies in CSF and serum pairs can be measured quantitatively using specialised ELISAs. A broad range of CE-marked CSF ELISAs for the detection of intrathecal antibodies is available from EUROIMMUN. The parameters encompass HSV-1/2, EBV-CA, VZV, measles virus, mumps virus, rubella virus, TBE virus, CMV, *Borrelia*, *Treponema pallidum* and *Toxoplasma gondii*.

The processing of the ELISAs is standardised with identical incubation schemes and exchangeable reagents. This enables easy parallel analysis of different parameters, as well as efficient automation. Results are quantified by means of 4- to 6-point calibration curves or using a single recalibrator with reference to a stored master curve generated by EUROLabCSF software. Use of a single calibrator increases the cost-effectiveness since fewer ELISA wells are required per analysis.

Automated quotient calculation

Specialised software simplifies the computation and interpretation of results. The EUROLabCSF software calculates the CSF/serum quotients for total IgG, specific IgG and albumin, as well as the limes quotient and AI. The results are displayed clearly in quotient diagrams according to Reiber and Lange. The software communicates bidirectionally between the EUROIMMUN ELISA processor or photometer, LIS and nephelometer, so that time-consuming and error-prone manual data transfer is no longer required.



Representation of intrathecal antibody synthesis

$$AI = \frac{Q_{spec}}{Q_{IgG}} = \frac{\frac{\text{Specific IgG in CSF}}{\text{Specific IgG in serum}}}{\frac{\text{Total IgG in CSF}}{\text{Total IgG in serum}}}$$

Calculation of the antibody index (AI)

Quality assessment

External quality assessment institutes such as INSTAND and ESfEQA offer programs for CSF diagnostics to help diagnostic laboratories to meet the high analytical standards required. These schemes cover a range of parameters such as measles virus, rubella virus, VZV, HSV-1/2, TBEV and *Borrelia*. In the INSTAND CSF schemes from October 2022, results obtained using EUROIMMUN CSF ELISAs matched the target values in 92 per cent to 99 per cent of the samples. This represented the highest pass rate of all tests used.

CXCL13 in neuroborreliosis

The chemokine CXCL13 is an additional CSF marker that can aid early identification of neuroborreliosis. High concentrations of CXCL13 in CSF are often detected before antibodies appear. CXCL13 determination can thus help close the diagnostic gap between infection and positive antibody test and identify neuroborreliosis at an earlier stage. CXCL13 is, moreover, useful for detecting reinfections, where intrathecal antibodies may be present from previous infection. CXCL13 also serves as a marker for therapy monitoring, as its concentration in the CSF sinks rapidly with successful treatment. CXCL13 can be determined by ELISA. EUROIMMUN offers the first CE-marked ELISA for this application.

Perspectives

Determination of the AI plays an important complementary role to PCR and other CSF analyses in the diagnosis of inflammatory CNS diseases. The antibody analysis can substantially increase the chances of identifying the causative agent of an acute CNS infection, enabling the implementation of potentially life-saving therapy.

Furthermore, the polyspecific MRZ antibody reaction is the most specific known marker for MS — a disease which often requires extensive differential testing before a diagnosis can be established. If no infectious cause for acute neurological symptoms is found, further differential diagnostics may focus on analysing autoantibodies against neural target antigens. This can help to delimit autoimmune encephalitis from CNS infections, which is critical due to different treatment regimens. ●

References

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