Detection of intrathecal antibody synthesis in CNS diseases

by Dr Jacqueline Gosink

The investigation of antibodies against pathogenic agents in cerebrospinal fluid (CSF) samples is part of the diagnostic work up in acute or chronic inflammatory processes of the central nervous system. This article describes the analysis of intrathecally produced antibodies in CSF and the interpretation of the results.

Central nervous system infections

Acute infections of the central nervous system (CNS) can be caused by viruses, bacteria, parasites or fungi. Although rare, acute CNS infections can be life-threatening emergencies requiring rapid diagnosis and treatment. Manifestations include meningitis, meningoencephalitis or encephalitis. Diagnosis can be challenging as patients may present with non-specific symptoms such as fever, headache and altered mental state. Cerebrospinal fluid (CSF) analysis is important for detecting inflammation of the nervous system and the meninges [1]. Alongside direct pathogen detection by PCR, investigation of intrathecally produced antibodies plays a crucial diagnostic role.

Viral CNS infections

Viral meningitis is the most frequent clinical presentation of CNS infections [2]. Enteroviruses are the most common causative viral agents followed by viruses of the *Herpesviridae* family. These include herpes simplex viruses (HSV), varicella zoster virus (VZV) and Epstein-Barr virus (EBV). Mumps virus is one of the main causes of viral meningitis in unvaccinated populations. Tick-borne encephalitis virus (TBEV) causes the severe and potentially fatal neurological disease TBE, which is endemic in parts of Europe and Asia and is growing in range and incidence. Further neurotropic viruses include cytomegalovirus (CMV), rubella virus and measles virus.

Neuroborreliosis

Neurological symptoms occur in around 2–4% of cases of Lyme disease, a seasonal tick-borne disease caused by human pathogenic *Borrelia* species. Neuroborreliosis has a favourable prognosis when diagnosed and treated early. According to the clinical practice guideline from the Germany Society of Neurology (DGN), detection of intrathecal synthesis of *Borrelia*-specific antibodies is one of the criteria that can secure a definite diagnosis of Lyme neuroborreliosis [3].

Neurosyphilis

Neurosyphilis can occur at any stage of syphilis, ranging from weeks to decades after initial infection with *Treponema pallidum*. Patients often have comorbidities such as HIV infection, which can complicate diagnosis and therapy. According to the guideline for neurosyphilis from the DGN, a diagnosis of neurosyphilis is secured when criteria for suspected neurosyphilis are confirmed by a local treponemal antibody reaction, measured by the detection of intrathecal production of antibodies against *Tre. pallidum* [4].

Multiple sclerosis

Some non-infectious chronic inflammatory diseases, such as multiple sclerosis (MS), trigger a polyspecific humoral immune response, leading to intrathecal production of antibodies against various non-causative agents. The detection of intrathecal antibodies against measles virus, rubella virus and/or VZV (MRZ reaction) is a specific indicator of MS and may help to differentiate MS from clinically similar diseases. A MRZ reaction is present at disease onset and can thus assist physicians in making early treatment decisions. MRZ testing in patients with suspected MS is recommended by the German Society for CSF Diagnostics and Clinical Neurochemistry (DGLN) in its current guidelines [5].

Determination of specific intrathecal antibodies

In the diagnosis of inflammatory diseases of the CNS, it is important to distinguish intrathecally produced antibodies from antibodies that have diffused from the blood into the CSF. This is done by measuring the concentrations of pathogen-specific antibodies and total immunoglobulin in both the CSF and serum of patients. The most important immunoglobulin class for detection of pathogen-specific antibodies in CSF is IgG.

When specific antibodies are formed intrathecally, their quantity in CSF in relation to total immunoglobulin increases, whereas in serum it stays the same (Fig. 1). Therefore, to detect intrathecal antibody production, a ratio is calculated: the quotient of pathogen-specific IgG in CSF to serum (Q_{spec}) in relation to the quotient of

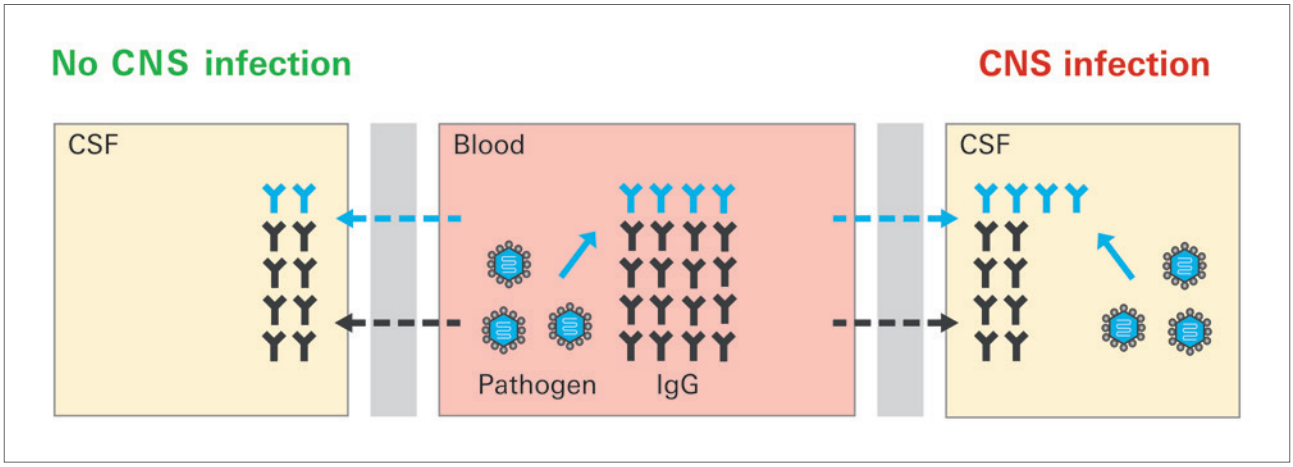

Figure 1. Diagrammatic representation of intrathecal antibody synthesis

total IgG in CSF to serum (Q_{IgG}) (Fig. 2). This is known as the antibody index (AI). In cases of elevated total IgG in CSF, the limes quotient, which is derived from the CSF/serum quotient for albumin, is used instead of the total IgG quotient for calculation of the AI [6].

AI values of 0.6 up to 1.3 are considered normal, values of 1.3 to 1.5 are borderline, and values of more than 1.5 indicate pathogen-s pecific antibody production in the CNS.

ELISAs for CSF/serum pairs

Specific antibodies in CSF and serum pairs can be measured quantitatively using specialized ELISAs. Ideally the CSF and blood samples should be collected at the same time (within one hour) of each other. A broad range of CE-marked CSF ELISAs for the detection of intrathecal antibody synthesis is available from EUROIMMUN. Tests for different parameters can be performed in parallel due to identical incubation schemes and exchangeable reagents. This standardized processing facilitates automation of the analyses. 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. The range of parameters encompasses HSV-1/2, EBV-CA, VZV, measles virus, mumps virus, rubella virus, TBE virus, CMV, *Borrelia*, *Tre. pallidum* and *Toxoplasma gondii*.

Automated quotient calculation

The complex computation of CSF/serum quotients and interpretation of results is fully automatically using dedicated software. The EUROLabCSF software calculates the CSF/serum quotients for albumin, total IgG and specific IgG, 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.

Quality assessment

Diagnostic laboratories can ensure the quality of CSF diagnostics through participation in external quality assessment schemes such as those offered by INSTAND and ESfEQA. These include 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% to 99% of the samples. This represented the highest pass rate of all tests used.

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

Figure 2. Calculation of the antibody index (AI)
Q_{IgG}, quotient of total IgG in CSF to serum;
Q_{spec}, quotient of pathogen-specific IgG in CSF to serum

>> Additional tests for neuroborreliosis

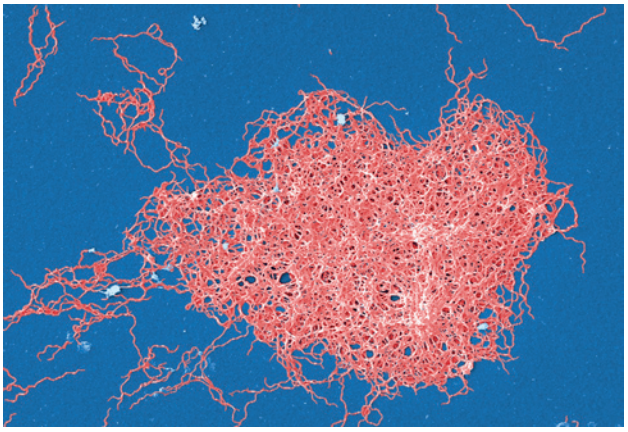
Line blots for use with CSF/serum pairs enable characterization of antibodies against a large spectrum of diagnostically relevant antigens. In contrast to serology, for which a two-tier strategy of screening and confirmation is recommended, the investigation of intrathecal antibodies by ELISA is sufficient in most cases. Immunoblots can nevertheless be useful as an additional test for neuroborreliosis diagnostics in cases of unclear or atypical findings. EUROLINE immunoblot profiles for CSF/serum analyses in borreliosis diagnostics encompass VlsE and OspC from different Borrelia species, p39, p41, p18, p19, p20, p21, p39, p41 and immunogenic lipids for highly differentiated antibody detection. Results are interpreted automatically based on the number and intensity of specific bands that react in CSF compared to serum. More bands and/or bands with a higher intensity in CSF compared to serum are an indication of intrathecal antibody synthesis.

The chemokine CXCL13 is an additional early marker of acute neuroborreliosis. High concentrations in CSF are frequently detected even before specific 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. However, it should be taken into account that increased CXCL13 values are also observed in some other CNS diseases. CXCL13 can be measured in CSF by ELISA. EUROIMMMUN offers the first CE-marked ELISA for this application.

Summary

Measurement of specific antibodies in CSF compared to serum is an important diagnostic tool to discriminate CSF- and blood-derived immunoglobulin and provide evidence of intrathecal antibody production in inflammatory neurological diseases. The presence of an MRZ reaction is an indicator of a polyclonal immune response in chronic inflammatory processes such as MS.

Further CSF tests used to support diagnosis of inflammatory neurological diseases include cell count, total protein, lactate, oligoclonal bands, cytology, CSF/serum glucose ratio and direct pathogen detection. Autoantibodies against different neural antigens can also be analysed in serum and/or CSF to investigate an autoimmune rather than infectious origin of the inflammation.



***Borrelia burgdorferi* is the pathogen responsible for Lyme disease and neuroborreliosis** (Shutterstock.com)

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