Cerebrospinal fluid analysis in the context of CNS demyelinating diseases

Análise do líquido cefalorraquiano no contexto das doenças desmielinizantes do SNC

Sandro Luiz de Andrade Matas¹,², Felipe von Glehn¹,³, Gustavo Bruniera Peres Fernandes¹, Carlos Augusto Senne Soares¹

ABSTRACT

The central nervous system (CNS) demyelinating diseases are a group of disorders with different etiologies, characterized by inflammatory lesions that are associated with loss of myelin and eventually axonal damage. In this group the most studied ones are multiple sclerosis (MS), neuromyelitis optic (NMO) and acute disseminated encephalomyelitis (ADEM). The cerebrospinal fluid is essential to differentiate between these different syndromes and to define multiple sclerosis, helping to assess the probability of Clinical Isolated Syndrome turn into multiple sclerosis.

Keywords: multiple sclerosis, acute disseminated encephalomyelitis, neuromyelitis optic, cerebrospinal fluid, clinical isolated syndrome, demyelinating.

RESUMO

As doenças desmielinizantes do sistema nervoso central são um grupo de desordens de diferentes etiologias, caracterizadas por lesões inflamatórias associadas a perda da mielina e eventualmente dano axonal. Neste grupo de doenças, as mais estudadas são a esclerose múltipla (EM), a neuromielite óptica e a encefalomielite aguda disseminada. O estudo de líquido cefalorraquiano é essencial para o diagnóstico diferencial entre as diferentes síndromes e para a definição de EM, ajudando a estimar a probabilidade da transformação da síndrome clínica isolada em EM.

Palavras-Chave: esclerose múltipla, encefalomielite aguda disseminada, neuromielite óptica, líquido cefalorraquiano, síndrome clínica isolada, desmielinizante.

The central nervous system (CNS) demyelinating diseases are a group of disorders with different etiologies, sometimes unknown, characterized by inflammatory lesions that are associated with loss of myelin and eventually axonal damage. In this group, because of the higher frequency, the most studied ones are multiple sclerosis (MS), neuromyelitis optic (NMO) and acute disseminated encephalomyelitis (ADEM), therefore, we will describe below the main cerebrospinal fluid (CSF) findings of each of these disorders.

Multiple sclerosis

Multiple sclerosis (MS) is a disease that affects 2.5 million people in the world and about 400,000 in the United States². MS is a primary disease of the central nervous system (CNS), clinically characterized by relapses mediated by acute inflammatory lesions in the white matter, followed by a progressive phase, mediated by axonal and neuronal loss.³⁴ The pathogenesis of MS is mainly driven by central nervous system-invading encephalitogenic CD4 T lymphocytes of both the Th1 and Th17 types. These effector cells can be downregulated by regulatory T lymphocytes.⁵ Current findings indicate that humoral immunity also plays a major role in disease pathogenesis, even though it is not fully understood.⁶ Intrathecal immunoglobulin synthesis in an oligoclonal pattern is the most common immunologic abnormality detected in MS patients.⁶⁷ These antibodies are produced against many different antigens, indicating localized B-cell expansion in brain, although a definitive association of these cerebrospinal fluid (CSF) antibodies with a consistent antigen has not been established.⁸ Oligoclonal bands (OCB) patterns differ between patients but remain constant during disease course.⁹ The frequency of CSF OCB differs between study populations. In Europe and in the USA, studies demonstrated more than 90% of frequency differing from the approximately 60% frequency in Asian studies.⁹¹⁰ In Brazil, a work involving 103...
MS patients demonstrated CSF OCB in 81% of the cases, not as high as northern studies. It was supposed that immigration mismatch has influenced the results. Different treatments have not modified CSF OCB IgG pattern, such as immunomodulators, immunosuppressors, rituximab and even complete immune ablation, such as in autologous hematopoietic bone marrow transplantation in severe cases of MS. However, it was recently demonstrated that natalizumab treatment can promote CSF OCB disappearance in some cases.

CSF OCB IgG is not specific for MS and could be also detected in some neuroinfectious and autoimmune diseases (e.g., neurosyphilis, Lyme disease, subacute sclerosing panencephalitis, ADEM), but because of the mentioned high frequency, its presence in association with the patient’s clinical presentation and neuroimaging data could confirm MS diagnosis, according to McDonald criteria. Furthermore, in the case of clinical isolated syndrome (CIS), the first relapse of a CNS demyelinating disease, the presence of CSF OCB can predict the conversion to clinical definite multiple sclerosis (CDMS). After a 3 years follow up of 192 patients with CIS, Bosca and colleagues showed that 60% of them evolved to CDMS. Ninety four per cent of these patients presented CSF OCB at the time of the first relapse, meaning that the association of CIS and their presence could increase to 9 times the risk of CDMS conversion. Masjuan and colleagues demonstrated that CSF OCB had 91.4% sensitivity and 94.1% specificity in CIS conversion to MS, which was superior to the prediction associated to abnormal brain MRI.

The CSF examination may show evidence of inflammatory activity during relapses as slight elevation of mononuclear white blood cells (pleocytosis) with cell counts varying between 5 to 50 cells/mm³ in one third of MS cases. Cell count above 50 is unusual and indicates that other diagnosis should be excluded. Also, slight elevation of protein levels varying between 45 to 70 mg/dl, with increased globulin to album ratio can be found in 40% of the cases.

Intrathecal immunoglobulin synthesis could be assessed qualitatively (OCB detection) and quantitatively (Immunoglobulin index). The identification of IgG-specific oligoclonal bands is performed with IgG-isoelectric focusing on agarose gel followed by immunoblotting. The patterns were interpreted qualitatively by comparing the presence or absence of OCB in CSF and serum. There are 5 patterns, described as follow: Pattern 1= no OCBs in CSF or serum; pattern 2=CSF-restricted OCBs; pattern 3=CSF-restricted OCBs and additional identical bands in CSF and serum (combination of patterns 2 and 4); pattern 4=identical OCBs in CSF and serum; pattern 5=monoclonal bands in CSF and serum. Only patterns 2 and 3 indicate intrathecal IgG synthesis.

CSF and serum concentrations for immunoglobulins and albumin are measured by nephelometry and analyzed within the same analytical series. The other immunoglobulins (IgA, IgM) could also be studied in the same way. The CNS does not produce albumin or immunoglobulin G (IgG) and their CSF level in normal conditions correspond to 0.5% and 0.25% from the serum, respectively. Therefore, CSF/serum albumin quotient, QAlb=Alb CSF [mg/l]/Alb Serum [g/l], is used to assess the blood-brain barrier (BBB) function. As the upper reference limit of QAlb is age dependent, Qlim(Alb) was calculated as 4 + (X/15), with X representing the patient’s age, according to Reiber’s study. Dysfunction of the blood-CSF barrier was defined as QAlb > Qlim(Alb). With this information, Tourtellotte developed quantitative expressions of the intrathecal humoral immune response based on calculation of the CSF/serum quotients (QIgG) with QIgG=IgGCSF [mg/l]/IgG Serum [g/l]. The upper limits of the respective reference ranges Qlim(IgG) is calculated against QAlb according to Reiber’s revised hyperbolic function. Values for QIgG exceeding Qlim(IgG), in this case IgG index values above 0.7, were considered to indicate intrathecal immunoglobulin synthesis. Reiber and Felgenhauer developed a diagram, which is divided in 5 specific areas: 1=normal value; 2=BBB disturbance without IgG intrathecal production; 3=BBB disturbance with IgG intrathecal production; 4=IgG intrathecal production without BBB disturbance and 5=Insignificant values.

Neuromyelitis optica

Neuromyelitis optica (NMO) is an inflammatory relapsing disease of the human central nervous system (CNS) of putative autoimmune etiology which is characterized by severe attacks of myelitis and optic neuritis (ON). In 60-80% of cases, NMO is associated with antibodies to aquaporin-4 (AQP4Ab), the most abundant water channel in the CNS, and its presence is related to a relapsing and often worse disease course.

Jarius and colleagues demonstrated a substantial lack of intrathecal AQP4-Ab synthesis in patients with NMO Spectrum Disorders (NMOSD). AQP4-Ab were detectable in 68% of CSF samples from AQP4-Ab seropositive patients with NMOSD, but in none of the CSF samples from AQP4-Ab seronegative patients with NMOSD. Therefore, they concluded that testing for CSF AQP4-Ab did not improve the sensitivity and specificity of the current diagnostic criteria for NMO and thus, its CSF measurement is unnecessary for clinical purposes.

CSF-restricted oligoclonal IgG bands, a hallmark of MS, are absent in most NMO patients, with the studies demonstrating around 15-30% of detection rate. If present, intrathecal IgG (and, more rarely, IgM) synthesis is low, transient, and, importantly, restricted to acute relapses. In addition, QAlb may be elevated both during relapse and during remission, indicating sustained blood CSF barrier dysfunction andsubclinical disease activity in patients with AQP4-Ab positive NMOSD.
CSF pleocytosis is present in around 50% of samples, with cell counts varying between 28 to 57 cells/mm³, sometimes reaching 2,000 cells/mm³. Frequently, differential cell analysis demonstrates neutrophils, eosinophils, activated lymphocytes, and/or plasma cells. Albumin CSF/serum ratios, total protein and CSF L-lactate levels correlated significantly with disease activity as well as with the length of the spinal cord lesions in patients with acute myelitis. Total CSF protein level is increased between 290 to 640 mg/dl during relapse and between 33 to 63 mg/dl at the remission phase. Additionally, CSF findings differed significantly between patients with acute myelitis and patients with acute optic neuritis at the time of LP.

**Acute disseminated encephalomyelitis (ADEM)**

Acute disseminated encephalomyelitis is an inflammatory demyelination disease of the CNS, encompassing the white and gray matter of the brain and spinal cord, with a distinct tendency to a perivenous localization of pathological changes. Children are mostly affected, with the median age of 6.5 years. Usually, ADEM is monophasic and associated with a temporal and probably also with a causative relationship to infection (e.g., measles, herpes, varicella, mumps, Epstein-Barr or influenza) as well as to preventive vaccination, although the development of vaccines that are based on recombinant proteins, which are not contaminated with undue amounts of myelin antigens, has significantly lowered its related incidence. Typically, the clinical syndrome begins with fever, headache, vomitus and meningeal signs, followed by consciousness and behavioral disturbances, seizures and focal neurological signs. CNS lesions are almost always of similar age, and consist of mostly one distinct pattern: perivenous and subependymal inflammation around small vessels in both white and gray matter, with infiltration of lymphocytes, macrophages and to lesser extent neutrophils. The topography of demyelinating lesions has a marked preponderance of the white matter at the cortical-subcortical border. Bilateral deep grey matter lesions (thalamus and basal ganglia) could also be affected. Highly intense infiltrates may also be found in the cerebellum, spinal cord and brainstem.

CSF examination is usually performed to rule out infectious meningoencephalitis and in the majority of cases, shows only minor and unspecific changes. In some cases, it may reveal a mild pleocytosis, with lymphocytes, monocytes and sometimes, plasma cells, usually between 10 to 50 cells/mm³, no more than 100/mm³. The total protein content is also increased but usually below 100 mg/dl. CSF OCB may be present only transiently and rarely, with the studies demonstrating a median of 12.5% of the cases studied. High serum titers of IgG specific for myelin oligodendrocyte glycoprotein (MOG) have been observed in 40% of the studied cases of ADEM.

**References**