Laboratorial Diagnosis of Lymphocytic Meningitis

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Meningitis is the main infectious central nervous system (CNS) syndrome. Viruses or bacteria can cause acute meningitis of infectious etiology. The term "Aseptic Meningitis" denotes a clinical syndrome with a predominance of lymphocytes in the cerebrospinal fluid (CSF), with no common bacterial agents identified in the CSF. Viral meningitis is considered the main cause of lymphocyte meningitis. There are other etiologies of an infectious nature. CSF examination is essential to establish the diagnosis and to identify the etiological agent of lymphocytic meningitis. We examined CSF characteristics and the differential diagnosis of the main types of meningitis.
Key-Words: Cerebrospinal fluid, lymphocytic meningitis, viral meningitis, meningitis.

Central Nervous System (CNS) infections classically are classified as meningitis and encephalitis [1]. Meningitis is the most common infectious CNS syndrome, defined as an inflammation of the meninges. The clinical symptoms are fever, malaise, vomiting, and in some cases, petechial rashes. Signs of meningeal irritation include neck stiffness, Kernig's sign, (an inflection of the knee when the limb is placed at a certain degree of relative inflection to the trunk), and Brudzinski's sign, (an involuntary inflection of the limb following a head inflection). These signs are poorly sensed in adults. In one study of adults, both Kernig and Brudzinski signs had a sensitivity of only 5%, while the sensitivity of nuchal rigidity was 30%. The non-specific nature of the symptoms and clinical signs means that we often over-treat and look to other exams to confirm the diagnosis [2].

Signs of meningeal irritation are rare among younger children. Small children can present other signs, such as an inability to feed, vomiting, drowsiness, convulsions, and a bulging fontanel. Table 1 presents the classification of meningitis in accordance with duration.

Encephalitis includes clinical signs of brain parenchyma involvement, fever, chronic headache, conscience alteration, which can be followed by focal neurological signals or seizures of recent onset. Meningoencephalitis occurs when meningitis is followed by involvement of brain parenchyma.

Acute meningitis with infectious etiology is viral or bacterial. From the first month of life, the bacteria *H. influenzae*, *N. meningitidis* and *S. pneumoniae* are responsible for 70 to 90% of the cases of acute bacterial meningitis in all regions of the world [3, 4]. Infections by *H. influenzae* have been significantly reduced because of systematic vaccination. The methods used for etiological diagnosis for acute bacterial meningitis are indicated in Table 2 [5-11].

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Table 1. Classification of meningitis

Type	Duration	Etiology
Acute	< 4 weeks	Infectious Non-infectious
Recorrent	Multiple acute Episodes, duration < 4 weeks	Infectious Non-infectious
Cronic	> 4 weeks	Infectious Non-infectious

Table 2. Etiological diagnostic methods for acute bacterial meningitis

Traditional bacteriologic methods
Direct bacterioscopy (Gram stain)
Cultures
Immunologic methods
Latex particles agglutination
Co agglutination
Counter immunoelectrophoresis (CIE)
ELISA
Polymerase chain reaction (PCR)

Chronic meningitis of infectious causes is caused by tuberculosis, syphilis, fungus (mainly *Cryptococcus neoformans*), cysticercosis and histoplasmosis, amongst other causes [12].

Aseptic means, according to the Oxford Dictionary, free from putrefaction or blood poisoning and absence of pathogenic germs. The term "Aseptic Meningitis" is related to the clinical syndrome of inflammation of the meninges, with predominance of lymphocytes in the cerebrospinal fluid (CSF), and no common bacterial agents in the CSF. The absence of signs of encephalic parenchyma involvement is implicit (encephalitis). Many authors consider the term aseptic meningitis to be synonymous with viral meningitis, although lymphocyte meningitis would be more appropriate. Though viral meningitis is the main cause of increased lymphocytes in the CSF, there are other etiologies of infectious nature (Table 3) [13-15].

Table 3. Etiology of lymphocytic meningitis

Infectious	Non-Infectious
Virus	Autoimmune diseases
Mycobacterium	Carcinomatous meningitis
Syphilis	Parameninges infection
Cryptococcus	Medicamentous
Lysteria	
Brucella	
Mycoplasma	
Neurocysticercosis	
Toxoplasmosis	
Leptospirose	

Diagnosis of Lymphocytic Meningitis

When there is clinical suspicion of meningitis, analysis of the CSF is mandatory. CSF examination is essential to establish the diagnosis and to identify the etiological agent. CSF characteristics of the main types of meningitis are indicated in Table 4 [8,16-18].

The preferential site for CSF collection is lumbar, at the level of the dural sack [19,20]. A cisternal puncture, at the level of the magna cistern, has restricted indications nowadays [21]. The only absolute indication for cisternal puncture is intracranial hypertension or dermic/epidermic infection in the lumbar region. The lumbar puncture must obligatorily be done when there is suspicion of a medullar process; in these cases, if the puncture is done at the subocciptal level, the CSF can remain without alterations. Another location for CSF collection is ventricular. Ventricular puncture is always a neurosurgical procedure; it is not the place of choice for CSF collection. It is mainly carried out in children with an open fontanel, in neurosurgical patients or in those with problems of ventricular shunt [14].

The best test to differentiate bacterial from viral meningitis is the CSF lactate test. Lactate levels are particularly important when CSF Gram staining is negative and there is a predominance of polymorphonuclear (PMN) cells, with low glucose in the CSF [20]. CSF lactate concentrations greater than 3.5 mmol/L are characteristic of acute bacterial meningitis. As the lactate concentration in the CSF is independent of that of serum, there is no necessity to collect matched serum. CSF lactate levels are also useful for the diagnosis of post-surgical acute-bacterial meningitis, when there is no specific increase in cells and proteins [22-24].

Lumbar puncture repetition is recommended for a patient with fever and chronic headache that does not disappear after some days, for patients with predominance of polymorphonuclear leukocytes, or low glucose in the CSF, or in case of a doubt in the initial diagnosis. As molecular biology studies of the CSF continue to improve and new diagnostic techniques become more readily available, the etiological agents of viral meningitis will be identified more frequently [25-27].

PMN predominance (50%) can occur within the first six hours of the onset of viral meningitis; after this time there is a change in the characteristics of the CSF to the typical pattern of viral meningitis [14,15,25,27,28].

Quantity of CSF Collected

To determine the quantity of CSF to be collected, the CSF analyses that will be requested (Table 5) must be considered. In general, 10 to 15 mL of CSF are collected for diagnostic purposes; 500 mL of CSF (0.4 mL/min) are produced per day. The average time for total renewal of the CSF is from four to four hours. The 10 to 15 mL removed will be renewed in about 30 minutes.

To look for alcohol-acid resistant bacilli (BAAR), fungi or neoplasic cells, 5 mL are necessary for each analysis. Moreover, more than three serial CSF punctures increase the sensitivity of such samplings.

The CSF total cell number must be analyzed as soon as possible, within at least two hours after the lumbar puncture. Cell destruction, precipitation and fibrin formation begin immediately. These can interfere significantly with cell counts. If the CSF will not be analyzed immediately, the sample must be kept under refrigeration [14]. Almost 40% of the WBCs are

Table 4. Normal CSF characteristics and main pathological alterations

		Meningitis		
Characteristics	**Normal**	**Acute bacterial**	**Viral**	**Chronic**
Pressure (mm/H_2O)	100-200	N or ↑	N or ↑	N or ↑
Aspect	Clear	Turbid/purulent	clear/cloudy	Clear/cloudy
Color	Clear	white	Clear	Clear/white
Cytology (mm^3)	Until 4	> 1,000	500-1,000	< 500
Cell type	Lymphocytes	Neutrophils	Lymphocytes	Lymphocytes
Protein (mg/dL)	V 5-10 SO 10-25 L 15-45	↑↑	Normal or ↑	Normal or ↑
Glucose (mg/dL)	2/3 from serum	↓	Normal	Normal or ↓
Lactic acid (mmol/L)	< 3.5	> 3.5	< 3.5	< 3.5

Table 5. Minimum volume of CSF necessary for routine analyses

Exam	(mL)
Complete CSF (glucose*, protein, global cytology and differential)	3
Protein Electrophoresis	5
Lactate	1
Immunological reactions	2
Direct bacterioscopy and cultures**	2

*Concomitant collection of serum glucose. **CSF culture must be collected in an appropriate tube with chocolate agar.

destroyed after two hours at room temperature and at 4°C 15% are destroyed. One hour after the collection there is a 32% reduction in the initial counts of neutrophils, and after two hours, 50% are lost. There is no significant reduction of lymphocytes or monocytes up to three hours after collection.

The normal number of WBCs in the CSF in adults varies from 0 to 3 cells/mm³, or according to some authors, a maximum of 5 cells/mm³ [14]. In children less than one year old, it varies from 0 to 30 cells/mm³; however, there is no absolute consensus on the normal values of CSF cells in children [15,29,30]. Normal CSF contains a small number of lymphocytes and monocytes. The reference values are indicated in Table 6. The lymphocytes present in the CSF are similar to those in the peripheral blood. Small lymphocytes predominate, and 75 to 95% are T lymphocytes [31].

Table 6. Normal differential CSF cytology

	Adults	Newborns
Lymphocytes	60 + 20%	20 + 15%
Monocytes	30 + 15%	70 + 20%
Neutrophils	2%	4%

Lymphocytic Meningitis with Infectious Etiology

Viral Meningitis

Viral meningitis is a worldwide disease, which can be either sporadic or epidemic. Despite the low mortality rates, there can be high morbidity [25]. The main viruses causing meningitis are indicated in Table 7.

Non-polio enteroviruses are responsible for most cases of viral meningitis (50% to 80%), especially during summer. Within the enterovirus group, there is an important further division into the Picornaviridae family: Echovirus [4,5,7,10,12,16,22,31], the Polioviruses and the Coxsackieviruses of groups A and B 1,2. Enterovirus numbers 70 and 71 show a strong neurotropism, which is associated with meningoencephalitis, polio-like paralytic syndromes, Guillain Barré Syndrome, as along with meningitis. Coxsackie virus sub-group B is responsible for 60% of cases of meningitis among children less than three years old [25].

Table 7. Virus responsible for lymphocytic meningitis

Common	Less frequent	Rare
Enterovirus	HSV-1	Adenovirus
Coxsackie virus A e B	LCV	CMV
Echovirus	Mumps	EBV
Arbovirus*		Influenza A, B
HIV		Measles
HSV-2		Parainfluenza
		Rubella
		VZV
		HHV-6

*The types of arbovirus are different depending on the area, it is important to investigate areas or countries visited by the patients. LCV=Lymphocytic choriomeningitis virus.

The viruses of the herpes family collectively are responsible for 4% of the cases of meningitis. Meningitis more frequently is caused by HSV-2; HSV-1, 2 and EBV are associated with recurrent lymphocytic meningitis [25].

Almost 5% to 10% of HIV positives have meningitis at any phase of the infection; however, it is more frequent during the seroconversion period. The ELISA test for anti-HIV antibodies in the CSF and in serum is generally negative in this phase. For diagnosis, the patients with suspicion of meningitis due to HIV must be followed and the anti-HIV ELISA of the serum repeated, or the CSF-HIV-viral load can be determined.

Parotid disease can be associated with meningitis in 10% to 20% of the cases; it is more frequent during winter months, and in male patients in a 3/1 ratio.

Laboratorial investigation of lymphocytic meningitis is shown in Table 8. The laboratory techniques for viral detention are: viral isolation in cell culture (done in reference laboratories) and detection of the viral genome using RT-PCR or PCR.

Specific antibody anti-virus detection, which can be useful, is in general gradually being substituted by molecular techniques of genome amplification (RT-PCR/PCR). Other clinical materials, such as feces, urine, and blood, can be analyzed in association with the CSF. However positivity of the reaction does not confirm CNS infection [32].

Herpes encephalitis, along with the infection for HSV-1, is diagnostically different from meningitis. The CSF is altered in 97% of the cases. However, there are no pathognomonic alterations. There is an increase in WBCs, from 5 to 500 cells/mm³, with a predominance of lymphocytes, moderate CSF total protein increase and normal or slightly reduced glucose [24,34]. The presence of red blood cells, in the absence of traumatic lumbar puncture, occurs in 40% of the cases and xantochromic CSF occurs in 11% of cases. These two CSF characteristics help to distinguish the diagnosis from other types of encephalitis [14]. The increase in the IgG levels occurs after the second week of illness. Specific levels of CSF anti-HSV IgG are elevated, and correspond to the increase in the serum. They can remain high three months to three years after the

Table 8. Laboratorial investigation of lymphocytic meningitis

LCR	Serum
RT-PCR to Enterovirus	Anti HIV
PCR to HSV2 DNA	VDRL/ FTA-ABS
PCR to EBV DNA	
PCR to CMV	
PCR to HIV-1 RNA	
VDRL	
ELISA to neurocysticercosis	
Direct research of BAAR*	
M. tuberculosis culture	
PCR to *M. tuberculosis*	
Capsular antigen to *C. neoformans*, latex**	
Histoplasmosis research	
Neoplasic cells	

Bacterial infections always must be ruled out. *10 mL to direct search. **_C. neoformans_ capsular antigen could be associated by latex agglutinations in the urine or serum.

acute illness [35]. Most of the patients have developed serum antibodies against HSV previously; therefore serological tests do not have a diagnostic value. A fourfold increase in serum antibodies does not represent sensitivity or specificity. There is intrathecal anti-HSV antibody synthesis against HSV; an increase of four times in these antibodies or an increase in the relation of anti-HSV antibodies in the CSF/serum have diagnostic value; however, this increase occurs slowly and is used as diagnostic confirmation retrospectively [36,37]. Detection of specific antibody anti-HSV can be calculated through the relation (antibodies index - AI) between the CSF/ serum quotients for the specific antibodies (Q spec) and the IgG quotient (Q IgG), AI = Q spec/Q IgG. Values greater than 1.5 indicate local synthesis of specific antibodies [32,38-40]. The polymerase chain reaction (PCR) in the CSF, to amplify the HSV DNA, is the method of choice for HSV diagnosis [38,41-43]. The PCR is positive 24 to 48 hours after the beginning of neurological symptoms and remains positive during two to five days after the beginning of treatment with antivirals.

The sensitivity of the PCR reaction depends on a series of factors. The sensitivity of PCR for HSV is 94%, the specificity is 98%, positive predictive value is 95% and negative predictive value is 98% [33,38,44-48].

There is a relation between the detection of virus by PCR in the CSF and the onset of neurological symptoms. The highest positivity of the PCR for enterovrrus occurs between the 3rd and the 14th day [49].

Neurosyphilis

CSF VDRL is the gold standard for the diagnosis of neurosyphilis; it has a sensitivity of 30% to 70% [50]. False positive results are described only in cases of traumatic lumbar puncture. A positive CSF VDRL establishes the diagnosis; however, if negative, the CSF VDRL does not exclude the diagnosis. The CSF FTA-ABS is 100% positive in cases of neurosyphilis and negative in 100% of the cases without syphilis; 23% of the cases with systemic syphilis are positive. The percentage of false positive results is as high as the serum FTA-ABS [51]. The CSF FTA ABS sensitivity is 100%, and the specificity varies from 39% to 89% [32,52].

Table 9. Sensibility and specificity of PCR for virus in the CSF

Virus	Sensibility (%)	Specificity (%)
HSV 1	> 95	100
CMV	80-100	75-100
VZV		100
EBV	97	100
JC virus	74-92	92-96
Enterovirus	97	100

Other causes of lymphocytic meningitis should be suspected, depending upon the region of origin of the patient or on his immunological status [53].

Lymphocytic Meningitis with Non-Infectious Etiology

Chemical Meningitis

Some intrathecal medicines, such as antibiotics, including metrotexate, anaesthetics, aracytin, baclofen, corticoids, or contrast chemicals, can cause chemical meningitis. The presence of blood in the CSF due to subarachnoid hemorrhage also can cause an increase in CSF cells and low glucose. Normal CSF does not have red blood cells. When there are red blood cells in the CSF, it is important to separate subarachnoid hemorrhage from traumatic lumbar puncture (Table 10).

Macrophages with red blood cells in their interior have no value to differentiate HAS from traumatic lumbar puncture, because macrophages persist with phagocytic activity *in vitro* more than six hours. Crenate red blood cells also are not important in the differential diagnosis. The first three criteria provide the differential diagnosis in almost 80% of the cases. A CSF with traumatic puncture has an increase in cells and proteins, due to the leakage of these elements from the blood. The correction of total CSF-cell number is done by following

Table 10. Differential diagnosis between subarachnoid hemorrhage and traumatic lumbar puncture

	HAS	Traumatic lumbar puncture
3 Tubes (Tuffier Millian)	same	Different
Supernatant post-centrifuge	Xantochromic	Clear
Clot	Absent	Present
Macrophages with hemosiderin	Present	Absent
Hemoglobin x bilirubin (%)	Bilirubin predominance	Hemoglobin predominance

the relation:

$$\text{Corrected WBCs} = \text{CSF WBCs} - \frac{\text{blood WBCs} \times \text{CSF RBC}}{\text{blood RBC}}$$

Practically, it is considered that each 700 to 1,000 red blood cells/mm³ increases 1 cell/mm³ in the CSF and 1mg/dL of total CSF protein. This correction is valid only for traumatic lumbar puncture; in the case of HSA there is increase in total CSF cells due to chemical meningitis caused by the presence of blood in the subarachnoid space.

Medicamentous Meningitis

The systemic use of some medicines, such as non-steroid anti-inflammatory drugs, antibiotics with sulfa, intravenous immunoglobulin, isoniazide, and Muromonab-CD3, can cause an increase of WBC in the CSF, with predominance of lymphocytes [14,54,55].

Carcinomatous Meningitis

The hypothesis of CNS involvement by malignant neoplasms must be examined in a patient with known malignant neoplasms and neurological symptoms. Malignant cells from a variety of tumors, metastatic or primary, can be detected in the CSF. Any type of neoplasm can spread to the leptomeninges. This dissemination occurs more frequently in acute hematological diseases, such as leukemia and lymphomas. Among solid tumors, the dissemination is more frequent with melanomas and breast or lung cancer. Among CNS primary tumors, tumor cells are more commonly found in the CSF in gliomas and medulloblastomas, due to their higher incidence and tendency to spread into the subarachnoid space. The frequency of CNS primary lymphomas has increased over time and is particularly high in patients with cellular-immunity alterations, such as HIV [56-59].

The positivity rate of detecting malignant cells in the CSF varies in the literature, but it is around 24%, and it is assumed to depend on several factors, such as histological confirmation, localization of CSF collection, and CSF-processing methodology [60-62]. The sensitivity of detecting malignant cells in the CSF changes in accordance with the type of neoplasm, and with anatomic location, as well as with meningeal involvement and its extension and the number of malignant cells in the CSF [67]. Primary cerebral tumors that exfoliated cells to the CSF were all located adjacent to the ventricle. In contrast, cells from tumors deeply localized in cerebral parenchyma are more difficult to find in the CSF [63-68].

In the specific case of lymphomas, some markers can help to differentiate the total cell number increase from an inflammatory reaction or a CNS infiltration. For example, interleukin-10 (IL-10), a B-cell growth and differentiation factor, is normally undetectable in the CSF. Systemic lymphoma cells produce IL-10. Elevated levels of interleukin-6 (IL-6), an inflammatory cytokine produced by B and T lymphocytes, have been found in infectious and noninfectious nonmalignant inflammatory disorders. Elevated IL-10 with an IL-10 to IL-6 ratio greater than 1.0 is a strong predictor of the presence of lymphoma cells in the CSF. Alternatively, an IL-10 to IL-6 ratio of less than 1.0 is characteristic of an infectious or noninfectious nonmalignant inflammatory disorder [57,69,70].

Methods with more sensitivity and specificity than cellular morphology are necessary to correctly identify malignant cells in the CSF. Although CSF cytology is useful, malignant cells are not detected in as many as one third of patients who have compelling clinical or radiographic evidence of neoplastic meningitis. Novel procedures are being tested that may enhance the early identification of malignant cells in the CSF. Currently, the diagnosis generally is made after the onset of neurological manifestations and heralds a rapidly-fatal course for most patients. Immunocytochemistry techniques, immunophenotyping and biochemical or immunological markers can help in this diagnosis [56,57,66,71-78]. The analysis of CSF biochemical and cellular characteristics, although not specific for the diagnosis of malignant involvement of the CNS, is important and can help with the diagnosis of CNS neoplasms, when associated with other clinical or biomarker characteristics.

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