

Neurological complications in dengue infection: a review for clinical practice

Complicações neurológicas na dengue: uma revisão para a prática clínica

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ABSTRACT

Dengue is an important global public health problem. The World Health Organization estimates that 2/5 of entire world population are in risk of dengue infection. Almost 50 millions cases occur annually, with at least 20 thousand deaths. The etiological agent of this acute febrile disease is a single-strand positive-sense RNA virus of *Flavivirus* genus. It is an arboviral disease transmitted by *Aedes* sp. mosquitoes (*Aedes aegypti* and *A. albopictus*). Most infected individuals present asymptomatic infection, but some may develop clinical signs. Therefore, a wide spectrum of illness can be observed, ranging from unapparent, mild disease, called dengue fever, to a severe and occasionally fatal dengue hemorrhagic fever/dengue shock syndrome. Currently, neurological manifestations related to dengue infections are increasingly been observed and appears as a challenge for medical practice. In this study the neurological complications of dengue infection will be reviewed, focusing a better understanding of the disease for the clinical practice.

Keywords: dengue, nervous system, diagnosis.

RESUMO

A dengue é um importante problema de saúde pública. A Organização Mundial de Saúde estima que 2/5 da população mundial encontra-se em risco de desenvolver a infecção. Cerca de 50 milhões de casos ocorrem anualmente, com ao menos 20 mil mortes. O agente etiológico desta doença febril aguda é um vírus RNA, do gênero *Flavivirus*. Este arbovírus é transmitido pelo mosquito *Aedes* sp. A maioria dos indivíduos infectados apresenta infecção assintomática, porém alguns desenvolvem sintomas clínicos. Estas manifestações podem variar desde uma doença inaparente, branda, conhecida como febre da dengue, até uma forma severa, sendo fatal em alguns casos como na febre hemorrágica da dengue/síndrome de choque da dengue. Atualmente, manifestações neurológicas associadas à dengue são cada vez mais frequentes, tornando-se um desafio na rotina médica. Neste estudo, as complicações neurológicas da dengue serão revisadas, com ênfase na melhor compreensão acerca da doença para a prática clínica.

Palavras-Chave: dengue, sistema nervoso, diagnóstico.

The dengue virus belongs to the *Flaviviridae* family. There are four closely related, but antigenically different virus serotypes: DENV1 to DENV4. An infection caused by one of them can cause dengue fever and, rarely, most severe form of the disease. The infection provides life-long protective immunity to the same subtype, but no immunity against the other three serotypes. Moreover, previous infection with a different subtype increases the chances of developing dengue hemorrhagic fever. This is due to a phenomenon called antibody dependent enhancement (ADE). In ADE, the heterotypic non-neutralizing antibodies form complexes with dengue virus, which infect mononuclear phagocytes with enhanced

efficiency. Consequently, a higher number of host cells are infected, improving viral replication, worsening the clinical signs. Thus, this may also contribute to the development of neurological and other complications¹.

The neurological manifestations are mainly associated with DENV-2 and DENV-3. These serotypes were found in cases of encephalitis, meningitis and myelitis^{2,3}. DENV-4 was also detected in brain cells by immunohistochemistry and in cerebrospinal fluid (CSF) of a patient with encephalitis⁴.

The present article highlights some important points that should be addressed when evaluating a possible case of neurological complication of dengue virus infection.

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Support: Fundação de Amparo à Pesquisa do Rio de Janeiro (FAPERJ), CAPES.

Conflict of interest: There is no conflict of interest.

Dengue and the nervous system

The association of dengue infection and neurological abnormalities was first described by Sanguansermsri and colleagues in 1976, in a patient presenting with encephalopathy⁵. Although rare (1-5% of dengue cases), the neurological involvement have been increasingly reported in dengue epidemics. Furthermore, the cases may be underestimated⁶. The neurological complication can occur in patients with few or no signs of previous dengue infection. The age patients range between few months to 79 years old, being more frequent in children. In most of the cases, the neurological manifestations appear between two and 30 days after the onset of the fever⁷. The neurological symptoms are diverse and related to the location of the lesion. Headache, alteration of consciousness, irritability, insomnia, seizures and focal neurological deficit associated with encephalitis, encephalopathy and stroke pictures are the most common symptoms observed during the acute dengue. Other neurological manifestations, such as motor deficit can occur in acute dengue infection in cases of myelitis and myositis, or during post-dengue stage, in patients with polyradiculoneuritis, encephalomyelitis, neuro-myelitis optica, polyneuropathy and mononeuropathy⁸.

The neuropathogenesis of DENV infection is still poorly understood. Viral and host factors may play an important role in the neurological disorders associated with Dengue. In this context, direct viral infection of central nervous system (CNS), autoimmune reaction, metabolic and hemorrhagic disturbances can be involved in the pathogenesis⁹.

Central nervous system DENV infection

The Dengue virus infection of the CNS has been confirmed by the detection of viral antigens and DENV RNA in brain tissue and cerebrospinal fluid (CSF) samples. The entry of the virus into the brain seems to occur through infiltrates of infected macrophages¹⁰.

The relation between viral factors and neuropathogenesis was demonstrated by Bordignon et al. (2007). The authors reported that a mutant DENV-1 caused an extensive leptomeningitis and encephalitis in mice. The hypothesis is that the mutant virus presented neurotropism, crossing the blood-brain barrier. Other evidence of DENV neurotropism is the detection of intrathecal synthesis of specific antibodies in patients with dengue myelitis¹¹.

Encephalitis, myelitis and meningitis are the most important neuroinvasive diseases associated with dengue. However, unlike other viral infections, meningitis due to dengue virus is rare, being more frequent in children. In these cases, the clinical manifestation is similar to other viral meningitis³.

Encephalitis is the most common neurological manifestation of dengue infection¹² and the main symptoms include seizures, altered consciousness, and headaches. Only half of individuals with encephalitis presents the classical

symptoms of dengue infection (myalgias, diarrhea, joint or abdominal pain, rash and bleedings). This fact contributes to the underestimation of cases. The diagnosis criteria for dengue encephalitis consists of: fever and acute signs of cerebral involvement associated with the presence of anti-dengue IgM or dengue RNA in the serum and/or CSF added to the exclusion of other causes of viral encephalitis and encephalopathy¹³. In these cases computed tomography (CT) and magnetic resonance imaging (MRI) findings may be normal. Encephalitis secondary to dengue infection usually has a benign outcome. However some patients exhibit persistent neurological symptoms¹⁴. Glucocorticoid can be used, which highlights the importance of an early diagnosis for a good prognosis¹⁵.

Myelitis associated with dengue infection is also uncommon (9.5% to 15%). It appears 7-30 days following the onset of dengue infection⁷. In these cases neurological symptoms, as paraparesis and sphincter dysfunction, may persist. Magnetic resonance imaging (MRI) usually demonstrates hyper-intense signal at the thoracic spinal level¹⁴.

Autoimmune reaction in DENV infection

Autoimmune reaction can occur after DENV infection as a result of the deposition of immune complexes in nervous system. Guillain-Barré (GBS) and Miller Fisher syndrome, neuromyelitis optica and optic neuritis and acute disseminated encephalomyelitis (ADEM) have been reported and related to immunological imbalance in dengue infection¹⁵.

The pathogenesis of ADEM associated with dengue suggests that the symptoms result from autoimmune reaction causing several areas of white matter brain lesions. Thoracic spinal cord can also be affected, showing demyelinating lesions¹⁵.

GBS was described in 30% of the neurological manifestations associated with dengue infection⁷. The clinical presentation of GBS cases induced by dengue is similar to those caused by other infections, with ascending paraparesis as the main manifestation¹⁶. Treatment is usually effective and the prognosis is good⁷. A case of Miller Fisher associated with dengue was reported presenting spontaneous recover¹⁷. The clinical manifestations of GBS result from cell-mediated immunological reactions. Dengue virus would initiate the immunological event and myelin or axons could be the target of this immune response¹⁸. In endemic areas, dengue infection should be investigated in cases of GBS⁷.

Finally, rare cases of fibular, ulnar, long thoracic, and phrenic neuropathy have also been related to dengue infection. Cranial palsy may also occur, mostly with oculomotor and facial nerves involvement⁹.

Benign acute myositis in dengue infection could be consequent of direct viral invasion and/or immune-mediated damage of muscle fibers¹⁸. In some areas, up to 50% of benign acute childhood myositis cases occur in dengue infection.

Myositis is considered a benign illness with a wide clinical spectrum, ranging from mild proximal asymmetrical weakness of the lower limbs to severe, rapidly progressing, limb, trunk, and respiratory muscle weakness. Severe fatal cases are not common. Serum creatine phosphokinase levels are always elevated. Muscle biopsies have revealed histopathological changes (perivascular mononuclear infiltrate, lipid accumulation, foci of myonecrosis, and an increase in mitochondria number)¹⁹.

DENV and metabolic disturbance

In acute phase of dengue infection, dengue shock syndrome (DSS), brain edema, cerebral anoxia, metabolic acidosis, electrolyte disturbances, vasculitis, liver and kidney failure can cause encephalopathy²⁰.

Hemorrhagic disturbances in DENV infection

In some cases of acute infection, brain hemorrhage has been reported. It is associated with thrombocytopenia and endothelial damage, with increased vascular permeability and subsequent leakage of fluid.

In both hemorrhagic and metabolic disturbances, pathological studies revealed non-specific lesions, edema, vascular congestion, and focal hemorrhages¹⁵.

Laboratory diagnosis of dengue infection

Dengue acute infection

The gold standard for the diagnosis of dengue infection is still the virus isolation in cell culture followed by identification using fluorescent antibody. However, this technique is not used in clinical routine²¹. In this context, the diagnosis of acute dengue infection is based on the detection of viral antigens, viral RNA or dengue specific antibodies in blood. The antibody detection (IgM and/or IgG) using enzyme-linked immunosorbent assay (ELISA) is the most widely used technique for dengue diagnosis. It has high sensitivity and specificity²².

However, some important issues have to be addressed. In a primary infection, for example, the immunoglobulin M (IgM) is the first to be synthesized and presents a higher titer. In these cases, serum IgM antibodies can be detected from the 5th to 30-60th days after the onset of symptoms, with 92% of sensitivity and 99% of specificity. On the other hand, the IgG appears in low titers at the beginning of symptoms and increases slowly at the end of the first week of the onset of illness. Thus, in recent infection, the detection of dengue IgM antibodies in serum or the increase of dengue IgG titer in paired samples can be used to confirm serological diagnosis. In contrast, in secondary infection, high levels of IgG are detectable even in the acute phase, responding with titers too high in the next two weeks. The kinetics of the response of IgM varies, appearing later during the febrile disease, often preceded by IgG. In some cases, however, IgM is not detected

in secondary infections²². Specific antibodies may also be detected by complement fixation technique, neutralization test, hemagglutination inhibition assay, or by an immunochromatographic test. The latter is being increasingly used for the rapid diagnosis of dengue infection²³.

Another point that should be emphasized is the strong cross-reactivity that occurs between members of the *Flaviviridae* family in indirect immunoassays. The interpretation of serological results can be impaired in cases of others flavivirus infection, such as St. Louis encephalitis, Japanese encephalitis, West Nile fever, and yellow fever. These conditions can also cause similar clinical manifestation²⁴.

The detection of non-structural 1 (NS1) viral antigen is used for diagnostic purpose too. This protein is present at the onset of early symptoms and may persist until the fourteenth day after infection, which makes it useful for the early diagnosis of infection. Commercial kits using different methods have been employed for this analysis, such as immunoassays like ELISA test²². The commercial methods used for NS1 antigen detection in serum and plasma presented 52% and 66% of sensitivity and 90% and 100% of specificity²³. Currently, an immunochromatographic commercial kit that simultaneously detects the presence of IgG, IgM and NS1 antigen is available.

Nucleic acid tests (NATs) as the reverse transcription followed by polymerase chain reaction (RT-PCR) are also used as a direct test. It can amplify and detect the genetic material of dengue virus. It's considered a more sensitive technique than virus isolation in cell culture. The sensitivity can vary from 93% to 100%, depending on the serotype evaluated. It needs to be performed during viremia. Thus, NATs test can be done early after the infection, before IgM and IgG become detectable. Some varieties of RT-PCR have been developed and presented good efficiency by detecting and differentiating four serotypes of dengue virus using the methodology of semi-nested RT-PCR multiplex. In this technique, a reverse transcription is done, followed by two rounds of PCR, using primers serotypes-specific in the second stage of the technique²⁵. Another type of NATs is the real time RT-PCR. This technique is also based on the reverse transcription followed by PCR. Recently, the Center of Disease Control (CDC) from United States developed the first Food and Drug Administration (FDA) approved real time RT-PCR for detection and typing of DENV nucleic acid in suspected cases in the USA²⁶. The real time RT-PCR can be also used as a quantitative test in order to determine the viral load. However, this is still an "in house" technique and a rigorous previous validation is necessary before the implementation in clinical routine²⁷.

Neurological disease associated with DENV infection: diagnosis

The presence of dengue IgM, viral antigens or virus RNA in patients with acute neurological symptoms is sufficient for

the diagnosis of neurological disease associated with dengue virus infection. However, this diagnosis may become difficult due the possibility of neurological manifestation in oligosymptomatic/asymptomatic dengue cases. In this context, blood tests, CSF analysis and MRI images contribute not only to confirm the diagnosis and to exclude other possible differential causes of neurological alteration. These complementary exams help to monitor the patients and to a better understanding of disease pathogenesis. Routine hematologic, biochemical, and liver function, tests for rheumatic diseases, B/C hepatitis, and human immunodeficiency virus-1 (HIV-1) should be carried out in all suspected patients. Routine CSF analysis include total and specific cell count, determination of protein and glucose/lactate concentration, a smear and culture for bacteria and fungi, an assessment of blood-CSF barrier function by albumin quotient (CSF/serum), detection of intrathecal synthesis of total IgG, and measurement of specific antibodies against syphilis, cytomegalovirus, Epstein-Barr, and herpes simplex viruses⁹.

Cerebrospinal fluid analysis in suspected patients

CSF examination contributes to the diagnosis of neurological disorders associated with dengue. However, CSF analysis may be normal. Nonetheless, this does not exclude the diagnosis of neurological manifestations associated with dengue, once up to 50% of patients with encephalitis may have normal CSF⁷. Notwithstanding, in those cases, the CSF analysis still is of utmost importance⁹. In this respect, the CSF findings and possible neurological manifestation are resumed in Table 1.

Regarding specific tests for dengue infection in CSF, specific antibody (IgM and IgG), RNA or viral antigen should be also evaluated. Antibodies can be detected in CSF in the early stages of dengue CNS infection up until 5-7 days after the onset of neurological symptoms^{7,28}. The dengue IgM detection by ELISA presented a high specificity (97-100%) but the sensitivity varied between 0% and 73%, depending on the method used. The absence of specific IgM detection in CSF, however, does not exclude dengue as the causative agent of

neurological disorder^{6,7,28}. It is important to note that the detection of specific IgG in CSF is not a useful diagnostic tool, since they may be due to a prior infection and they can cross the blood-CSF barrier⁹. In relation of intrathecal synthesized dengue specific antibodies, previous study demonstrated that this is a potential marker of myelitis associated with dengue infection²⁹.

The detection of NS1 antigen in the CSF of dengue patients by ELISA exhibited a sensitivity of 50% and specificity of 100%. The combined use of these two markers (NS1 Ag and specific IgM) in CSF increases the sensitivity of dengue diagnosis to 92%²⁸.

The detection of dengue viral RNA in CSF, using PCR techniques presented variable results (0-83%)^{2,6,28}. This discrepancy may be associated to different viral phenotypes which may present distinct neurovirulent and neuroinvasive properties. Other factor that may affect the result includes the stage of the disease, once the viral RNA is detected only in acute phase. It is also important to remind that, as described before, not all neurological complications associated with dengue are caused by the direct virus neuroinvasion. Thus, some cases won't present viral RNA in CSF. Hence, the absence of viral RNA detectable in CSF do not exclude the diagnosis of neurological manifestation due to dengue infection. In a previous study, detection of viral RNA (multiplex RT-PCR) and quantification of viral load (real time RT-PCR) in CSF and serum samples from dengue patients with neurological manifestation were evaluated. Discordant results were found in seven patients. Three individuals with dengue RNA in the CSF had negative serum samples obtained on the same day as the CSF and four individuals presented viral RNA in serum but not in CSF. These findings emphasize the importance of CSF analysis. Moreover, three individuals with positive result in multiplex RT-PCR in CSF did not present RNA amplification in real time RT-PCR. In contrast, the viral load was determined in two patients with negative multiplex RT-PCR. More studies are needed before the implementation of the detection and determination of viral load in CSF in routine laboratory².

Table 1. CSF findings in different neurological manifestation associated with Dengue infection.

CSF analysis	Neurological manifestation	
Inflammatory CSF: Pleocytosis, hyperproteinorhachia, blood-CSF barrier dysfunction (albumin quotient $\geq 8 \times 10^{-3}$), and intrathecal synthesis of total IgG (IgG index ≥ 0.7 or oligoclonal IgG bands)*	Encephalitis Myelitis Meningitis	Neuromyelitis optica ADEM
Protein-cytological dissociation	Guillain Barré Miller Fisher syndrome	Polyneuropathy Mononeuropathy
Hemorrhagic CSF	Cerebro-meningeal hemorrhage	
Intrathecal synthesis of dengue antibodies**	Myelitis	
Detection of dengue IgM antibodies	Several neurological manifestation	
Detection of viral antigens and/or viral RNA**	Encephalitis Myelitis Meningitis	

*: indicates inflammatory reaction in CNS; **: indicates viral neuroinvasion.

As the virus is an arbovirus transmitted by *Aedes* mosquitoes, some environmental and vector characteristics are important to the disease emergence. Several factors contribute for the increase of density and geographic distribution of the vector, enhancing the virus transmission. Among them, temperature, pluviosity, living conditions, poverty, social inequalities and lack of knowledge about disease prevention constitute determining factors for dengue transmission. It is important to highlight that as increases the virus circulation and transmission, increases the probability of

severe clinical manifestation as nervous system involvement, mainly in areas with distinct serotypes circulation. Neurological complications in dengue infection become increasingly important as the dengue epidemics continue to occur. The clinical manifestations are diverse and results from different neuropathogenic mechanisms. To obtain a reliable diagnosis is of utmost importance to know the disease physiopathology. The development and validation of new diagnostic tests is important, especially regarding the NATs applications in CSF.

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