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Clinical and laboratory diagnosis of Zika fever: an update

Diagnóstico clínico e laboratorial da febre pelo vírus da zika: uma atualização

Analúcia R. Xavier; Salim Kanaan; Ronielly P. Bozzi; Luiza V. Amaral

Universidade Federal Fluminense (UFF), Rio de Janeiro, Brazil.

ABSTRACT

Zika fever can be defined as an acute febrile viral illness, mainly transmitted by the mosquito of the genus *Aedes*. It makes a differential diagnosis from diseases caused by other flaviviruses, such as chikungunya and dengue fever. Many people with Zika virus (ZIKV) infection will not have symptoms or will only have mild clinical symptoms. The clinical conditions are nonspecific and characterized by low-grade fever, pruritic erythematous maculopapular rash, non-purulent conjunctival hyperemia without pruritus, arthralgia, myalgia, and headache. It is a benign, self-limiting and short-duration condition. Complications such as Guillain-Barré syndrome (GBS), spontaneous abortion and fetal malformations, mainly microcephaly and retinal lesions, may occur. The laboratory investigation is more important in cases suspected of ZIKV infection that have evolved with neurological complications, in pregnant women, abortion or congenital malformations, and is a key part for diagnostic definition. Real-time polymerase chain reaction (RT-PCR) can detect the virus in blood samples about four to seven days after the onset of symptoms. In urine, it is possible to identify viral ribonucleic acid (RNA) up to 15 days after clinical onset, even if viremia has ceased, and it is an alternative for late diagnosis. Serological tests may also be performed, while there may be cross-reactivity with other flaviviruses. Immunoglobulin class M (IgM) can be screened between the 2nd and 12th week after clinical presentation. The immunoglobulin class G (IgG) can be identified after the 15th day, and is present even in the convalescence and cure phase. Non-specific laboratory abnormalities, in general, do not present significant alterations. Patients with GBS must have cerebrospinal fluid (CSF) collection for analysis.

Key words: *Aedes*; Flavivirus; arbovirus infections; polymerase chain reaction; laboratory test.

INTRODUCTION

Zika fever is a viral disease, mainly transmitted by a mosquito of the genus *Aedes*. Clinical manifestations include intermittent fever, pruritic erythematous maculopapular rash, non-purulent conjunctival hyperemia without itching, arthralgia, myalgia, and headache. Those symptoms disappear spontaneously within 3 to 7 days. The Zika virus (ZIKV) is a single-stranded ribonucleic acid (RNA) virus and has two lineages: one African and one Asian. It belongs to the genus *Flavivirus* of the family *Flaviviridae*⁽¹⁾.

ZIKV was first isolated in 1947 from a Rhesus monkey in the Zika forest of Uganda. Human cases were detected in 1952^(2,3). Since then the virus has spread in more than 14 countries, reaching

Africa, America, Asia and Oceania. The first outbreak of ZIKV infection reported was in 2007 in the South Pacific Island, Yap Micronesia. Since then, several cases were reported there and in other countries in Oceania and Asia^(4,5).

In Brazil, the presence of the virus was confirmed by the Health Surveillance Secretariat, Ministry of Health, in the northeast region (Bahia), in May 2015⁽⁶⁾. Brazilian sanitarians believe that the presence of the virus in the country may be the result of the great amount of tourists that came to the country during the FIFA Soccer World Cup in 2014, held in several states of Brazil. The primary form of transmission is by the bite of an infected female mosquito of the genus *Aedes* (*aegypti*, *luteocephalus*, *africanus*, *apicoargenteus*, *furcifer* and *nitatus*). In Brazil, *Aedes aegypti* is responsible for the urban transmission. It gets infected when it bites a sick person, as well as it happens with

yellow fever, dengue and Chikungunya diseases. After ingesting blood containing the virus, the mosquito needs about 10 days to become a vector and infect new hosts. Mosquitoes such as *Aedes aegypti* lay their eggs in and around standing water. Their development usually occurs at temperatures between 30°C-32°C. Rarely, eggs develop at temperatures below 16°C. That's the reason why tropical and subtropical regions are more favorable to keep the transmission cycle by the *Aedes aegypti*. Transmission is more frequent during the day, and is possible to occur both indoors and out.

ZIKV disease is not contagious, but there are reports of perinatal, sexual and blood transfusion transmission. The virus may be detected in several biological fluids: urine, semen, saliva, and breast milk. Although viral particles are found in breast milk, saliva and urine, currently there is no evidence of infection through these biological fluids. In a study carried out with mothers infected by ZIKV in Polynesia, viral RNA was detected in breast milk, but it was not possible to replicate the virus particles in cell culture. Breastfeeding should be encouraged once it brings great benefits to the child. Besides, there is a lack of evidence of a possible transmission of ZIKV through breast milk. On the other hand, vertical transmission of ZIKV, both intrauterine and during delivery has been documented^(7,8).

Sexual transmission has been questioned. There is only one case in the world of proven sexually transmitted virus, an uncommon event. The study reported the infection, confirmed by serology, of a woman after having sexual intercourse with her husband, who was infected by ZIKV on a trip. Although the virus was not investigated in the semen at that time, the study showed that it was possible to detect ZIKV RNA in the semen, although it was not detected simultaneously in the blood. This leads to believe that there might exist sexual transmission of the virus^(9, 10). There are reports detecting the virus in the semen within 30 to 40 days of the acute clinical symptoms of the ZIKV infection. ZIKV stays in women secretions for around 11 days. In the first half of 2016 the Center for Disease Control and Prevention (CDC) published recommendations for men suspected of ZIKV infection in order to prevent possible sexual transmission of the virus⁽¹¹⁾.

Zika is self-limited and benign in most cases. The signs and symptoms are similar to dengue and Chikungunya diseases, but in a milder clinical form (fever, conjunctivitis, photophobia, rash, myalgia and joint pain).

Laboratory diagnosis is fundamental for the definitive diagnosis. ZIKV infection may be detected in blood and urine tests, based on indirect techniques such as enzyme-linked

immunosorbent assay (ELISA), indirect immunofluorescence and immunochromatography, with determination of circulating antibodies. Also, it is possible to detect the ZIKV through a molecular methodology.

CLINICAL DIAGNOSIS

After the patient is bitten by an infected *Aedes* mosquito, it has an incubation period of around three to 12 days, and they may present symptoms of the disease^(12, 13). About 18%-20% of people infected with the virus develop the disease. There are reports of positives laboratory tests for ZIKV infection in asymptomatic people^(14, 15).

The most common signs and symptoms are: conjunctivitis, rash, sore throat, fever, joint pain, myalgia, and headache. The less common symptoms include: abdominal pain, constipation, diarrhea, dizziness, canker sore, photophobia, nausea, vomiting, anorexia, and retro-orbital pain.

In the initial stage of the disease the patient present states of fever between 38°C-38.5°C, which disappears in one or two days after the onset of the rash and headache. There is a skin maculopapular rash, which consists of small and multiple papules that may come together and form large red spots, they are often pruritic and with discrete relief. It mainly affects the face, neck, trunk and limbs, including the palm and sole. There is an improvement of the rash in two or three days and it disappear in one week, on average, but it may persist for two weeks⁽¹⁶⁾. Often, conjunctivitis that manifests as a hyperemia and edema with no eye purulent secretion is reported.

Patients present joint pain and myalgia and discreet low-back pain is less intense. The joints most affected are: hands, knees and ankles. The duration of the joint symptoms is around three to five days.

The disease is benign, self-limited and of short duration, but complications such as Guillain-Barré syndrome (GBS), of neurological origin, may result in the loss of progressive and temporary muscle strength. It has been reported, especially in Brazil, the relation of microcephaly and Zika fever during pregnancy. The greater likelihood of developing this malformation occurs when maternal infection happens in the first trimester of pregnancy. However, there is a possibility, even though to a lesser extent, of occurring in the second trimester. An infection in the last trimester of pregnancy has small risk of developing microcephaly, because the fetus is already fully developed.

LABORATORY DIAGNOSIS

Nowadays, it is fundamental to order laboratory tests, which use technologies in the determination of high sensitivity, specificity and predictive value tests. It helps us to make diagnostic decisions. The clinical laboratory is important, mainly to diagnose pathologies that occur in asymptomatic patients, as in neoplasia (cervical cancer) and viral diseases, as infection by the human immunodeficiency virus (HIV).

The physician analyzes the clinical history and physical examination to order, select and interpret the laboratory tests. Laboratory tests to diagnose ZIKV infection may be performed in blood, urine, semen, amniotic fluid and cerebrospinal fluid (CSF)^(7, 9, 17-19).

The molecular tests are direct; they determine the multiplication of viral particles (RNA) and amplify the genetic material to find the pathogen. The polymerase chain reaction (PCR) can detect the virus at the beginning of the disease, around four to seven days after the onset of symptoms. Acute infections by ZIKV can be detected by real-time (RT) PCR within the first seven days after the onset of symptoms, but it has also been detected in patients on the 11th day of illness^(17, 20).

The ZIKV was detected in a urine sample around 15 days after infection through the same technique of RT-PCR⁽¹⁸⁾. When detecting viral RNA in the urine after viremia, it suggests that urine testing may be a tool for late diagnosis of infection, as dengue virus⁽⁹⁾. If the result is negative on blood and urine samples, the possibility of infection can not be ruled out by the RT-PCR test. In cases of clinical suspicion in endemic areas, it is necessary to perform the antibody screening, when the RT-PCR does not confirm the infection.

According to the United States Centers for Disease Control and Prevention, screening for ZIKV in children with a potential risk of congenital infection is recommended for: 1) children with microcephaly or intracranial calcifications born to mothers who travel or reside in the transmission areas of the virus during pregnancy; 2) children born to mothers with positive or inconclusive tests for ZIKV infection. Congenital ZIKV infection is established when RNA or viral antigen is detected in the amniotic fluid, umbilical cord, placenta, or in neonate blood samples⁽⁷⁾.

In the ZIKV outbreak in Polynesia, it was observed that some individuals with the symptoms of the virus infection did not have a positive RT-PCR test in the blood collected in the first week of

infection. The same study tested blood and saliva samples from patients with symptoms of infection with ZIKV. As a result, saliva sample is preferred than blood sample for viral RNA detection ($p < 0.0001$). Detection of viral RNA from saliva was not associated with any specific clinical manifestation; however it was more identified on the third day of infection. It was recommended, in the acute phase of the disease, to collect blood and saliva samples to increase the sensitivity of the molecular detection of ZIKV. If blood sampling is not possible, only saliva should be considered. At the later stage of the disease, it is interesting to add viral RNA search in the urine sample⁽¹⁹⁾.

The search for circulating antibodies could be performed by different techniques such as ELISA, indirect immunofluorescence and by rapid immunochromatography test. In these indirect methodologies we have the possibility of cross-reactions with antibodies generated in response to an earlier infection by viruses of the same family, especially flavivirus of yellow fever and dengue. These tests are performed on the blood of suspected patients. In order to obtain a better interpretation, we must consider the ability of the test to be positive in truly sick patients and the ability of the test to be negative in people who do not actually have the disease (specificity). In Brazil, the National Sanitary Surveillance Agency [Agência Nacional de Vigilância Sanitária (Anvisa)] recorded kits for serology for Zika with 96.6%-96.8% sensitivity and 100% specificity.

Immunoglobulin class M (IgM) antibody detection characterizes acute infection. It must be performed around the third day of infection and may be detected between the 2nd and 12th weeks of the supposed exposure in endemic areas. If the test result is negative, the infection can be ruled out. It is also possible to quantify the Immunoglobulin class G (IgG), present in both convalescent and healing stages⁽⁴⁾. **Figure 1** shows a

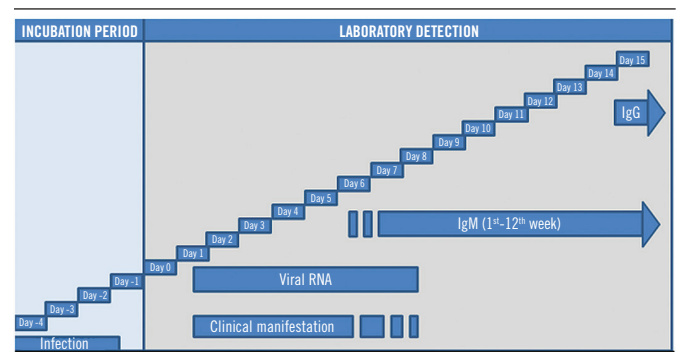


FIGURE 1 – Laboratory testing to detect ZIKV by serology (IgM and IgG) and RT-PCR versus days of infection (adapted from the Brazilian Ministry of Health)

ZIKV: Zika virus; RNA: ribonucleic acid; IgM and IgG: immunoglobulin class M and G; RT-PCR: real-time polymerase chain reaction.

temporal correlation between the clinical manifestations of the disease and the laboratory tests (molecular and serological).

Non-specific laboratory abnormalities, in general, do not present significant alterations. In this case it is possible to detect discrete leucopenia and thrombocytopenia. It is also possible to detect increased lactate dehydrogenase (LDH), gamma-glutamyltranspeptidase (gamma-GT), and inflammatory markers such as C-reactive protein (CRP), ferritin and fibrinogen⁽²¹⁾ in biochemical parameters.

In patients with GBS, it is important to collect and analyze the CSF, which will show a significant increase in proteins, with normal or little altered cellularity (albumin-cytological dissociation). This change is most significant in the second week of the disease progression, but does not exclude the possibility of being detected in the first week. Proteins reach their highest levels around the 4th and 6th weeks after the disease onset⁽²²⁾.

It is important to emphasize that only the laboratory tests can differentiate, with certainty, the ZIKV of the other flaviviruses that cause other acute febrile diseases, such as dengue and Chikungunya virus, mainly. Laboratory investigation is more important in cases of suspected ZIKV infection that have evolved with neurological complications, or in cases of pregnant women, abortion or congenital malformations. It stands out as a population at risk of ZIKV infection especially pregnant women in the first trimester. **Figure 2** provides an overview of what should be investigated in the cases suspected of ZIKV that require diagnostic confirmation⁽²³⁾.

CONCLUSION

The occurrence of ZIKV infection in areas already considered epidemic has shown a remarkable growth during this last decade. The disease presents a benign nature, but can evolve with complications of great morbidity and mortality, such as GBS and fetal malformation, when a pregnant woman is infected. The disease is spread by the mosquito *Aedes aegypti*, a common vector for other flaviviruses, which makes it possible to co-infect and hinders the differential diagnosis among viruses. In addition to the similar clinical condition and cross-reactions in serological tests, another point that makes diagnosis difficult is the low availability of laboratory test kits in the Brazilian market.

The contribution of laboratory diagnosis is of fundamental importance in the definitive diagnosis. The use of clinical laboratory tests in diagnosis of ZIKV infection can be performed

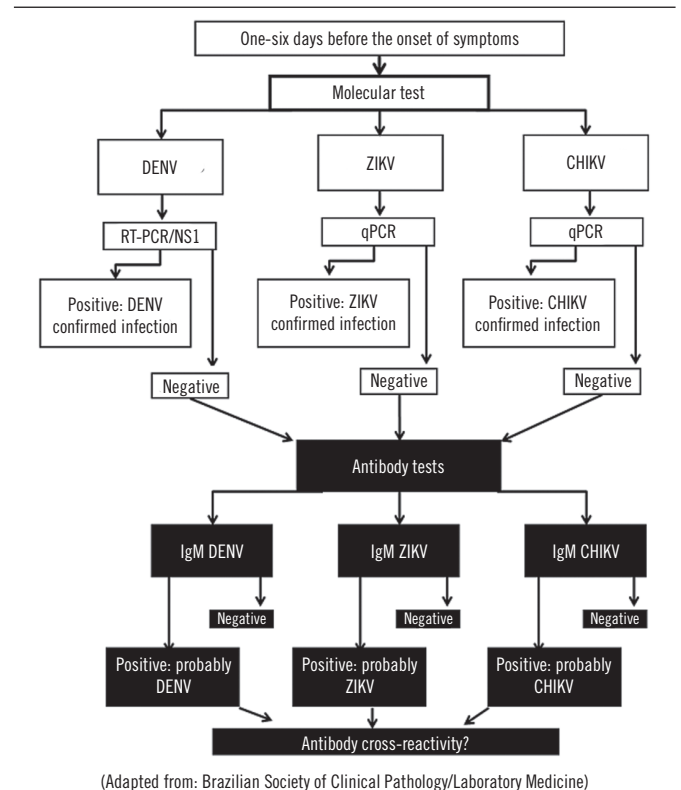


FIGURE 2 – Flowchart for laboratory testing to detect arbovirus in suspected cases of DENV, ZIKV and CHIKV

ZIKV: Zika virus; DENV: Dengue virus; CHIKV: Chikungunya virus; RT-PCR: real-time polymerase chain reaction; qPCR: quantitative polymerase chain reaction; NS1: nonstructural protein; IgM: immunoglobulin class M.

in blood, urine, semen, amniotic fluid and CSF. ZIKV infection can be detected in blood and urine tests, based on indirect techniques such as ELISA, indirect immunofluorescence and immunochromatography, with determination of circulating antibodies. There is still the possibility of detecting the infection through a molecular methodology. Detection of the viral RNA in the urine can be performed even after the viremia phase, and is a late possibility to detect the infection.

The most important measure for disease control is prophylaxis, especially with vector control, since the treatment is limited to clinical and symptomatic support, and there is no specific approach against the virus. There is also no vaccine against ZIKV. It is the obligation of the competent organs of the State to take more intensified vector control measures in suspected areas and places where there is circulation of mosquitoes of the genus *Aedes*. Assistance should also be provided to people with suspected or confirmed illness. A control action plan should also be drawn up to serve travelers in suspect areas.

RESUMO

A febre zika pode ser definida como uma doença viral febril aguda, transmitida principalmente pelo mosquito do gênero Aedes, e faz diagnóstico diferencial com doenças causadas por outros flavivírus, como a dengue e a Chikungunya. Muitos indivíduos infectados pelo vírus da zika (ZIKV) não terão ou terão apenas leve sintomatologia clínica. O quadro clínico é inespecífico e caracterizado por febre baixa, exantema maculopapular pruriginoso, hiperemia conjuntival não purulenta e sem prurido, artralgia, mialgia e cefaleia. A doença é autolimitada, de curta duração e de evolução benigna, mas podem ocorrer complicações, como síndrome de Guillain-Barré (SGB), aborto espontâneo e malformações fetais, principalmente microcefalia e lesões retinianas. A investigação laboratorial apresenta maior importância nos casos suspeitos de infecção pelo ZIKV que evoluíram com complicações neurológicas, em gestantes, aborto ou malformações congênitas, sendo fundamental para definição diagnóstica. A reação em cadeia da polimerase em tempo real (RT-PCR) pode detectar o vírus em amostras de sangue por volta de quatro a sete dias após o início dos sintomas. Na urina, é possível identificar o ácido ribonucleico (RNA) viral até 15 dias após o início do quadro clínico, mesmo que a viremia tenha cessado, sendo uma alternativa para o diagnóstico tardio. Os testes sorológicos também podem ser realizados, embora haja possibilidade de reação cruzada com outros flavivírus. A imunoglobulina da classe M (IgM) pode ser pesquisada entre a 2ª e 12ª semana após o início do quadro clínico; a da classe G (IgG), após o 15º dia, estando presente mesmo na fase de convalescência e na cura. As alterações laboratoriais inespecíficas, em geral, não apresentam alterações significativas. Nos casos em que os pacientes apresentam SGB, é importante a coleta e a análise do liquor.

Unitermos: Aedes; flavivirus; infecções por arbovirus; reação em cadeia da polimerase; testes laboratoriais.

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CORRESPONDING AUTHOR

Analúcia Rampazzo Xavier

Hospital Universitário Antônio Pedro; Departamento de Patologia; Rua Marquês de Paraná, 303, sala 4, 4º andar; Centro; CEP: 24033-900; Niterói-RJ, Brasil; Phone: +55 (21) 2629-9111; e-mail: analuciavaxavier@id.uff.br.