THE GROWING GLOBAL THREAT OF ZIKA, DENGUE AND CHIKUNGUNYA VIRUSES

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Arboviruses, or arthropod-borne viruses, are on the march globally. Increased urbanisation and international travel facilitate the spread of mosquito vectors and hence the viral diseases they carry. Zika virus (ZIKV) is currently spreading uncontrollably in the Americas, while dengue virus (DENV) and chikungunya virus (CHIKV) have already become firmly established in most tropical and also many non-tropical regions.

ZIKV, DENV and CHIKV infections are difficult to tell apart, as they manifest with similar clinical symptoms of fever, exanthema and arthralgia and are epidemic in much the same geographic regions. Therefore, laboratory analyses play an important role in differential diagnostics. Serological tests provide a longer diagnostic window than other methods such as direct detection, and are suitable for diagnosing acute infections as well as for disease surveillance. ELISA and indirect immunofluorescence test (IIFT) systems based on optimised antigens enable sensitive and specific detection of anti-ZIKV, DENV and CHIKV antibodies in patient serum or plasma samples.

Mosquito Vectors

Among the most important arthropod disease vectors in tropical regions are the species Aedes aegypti (yellow fever mosquito) and Aedes albopticus (tiger mosquito). These mosquitoes are active day and night and thrive in urban areas, making it difficult for humans to avoid bites. Their larvae multiply in open water reservoirs such as wells, cisterns and cloacae, as well as in small containers where rainwater collects.

Viruses spread by Aedes mosquitoes include members of the family of togaviruses and the family of flaviviruses. Due to their dramatic spread, ZIKV, DENV and CHIKV are among the most significant arboviruses. They are responsible for outbreaks of febrile infectious disease in Asia, Africa and the Americas, and it is feared that they could spread to other regions, including Europe. Aedes albopictus has already established itself in more than 12 southern and central European countries. Arboviruses are also reported to be endemic in the MENA region, although their epidemiology is not well characterised.

There are no antiviral therapeutics for these sometimes life-threatening diseases, and patients with complications have to undergo intensive medical treatment. A vaccine against DENV is in development, but vaccines against ZIKV and CHIKV are unlikely in the near future. The current most effective prophylaxis is individual protection from mosquito bites.

Zika Virus

ZIKV is a member of the family of flaviviruses and was first discovered in the Zika forest in Uganda in 1947. Until 2015 Zika fever was considered an obscure tropical disease, with sporadic outbreaks in countries in Africa and Asia and more recently on Pacific islands. In March 2015, first infections were reported in Brazil, and the virus has since spread rapidly throughout South America, Central America and the Caribbean.

In most cases the disease symptoms are mild. After an incubation period of five to 10 days, a flu-like illness develops with fever, rash, arthralgia, myalgia, headache and conjunctivitis. Zika virus infection has been linked to complications including congenital malformations, in particular microcephaly, and neurological conditions such as Guillain-Barré syndrome.

Chikungunya Virus

CHIKV belongs to the family of togaviruses. Chikungunya fever was first reported in Tanzania in 1952. The virus is now present in over 60 countries in Asia, Africa, Europe and the Americas.

The term chikungunya derives from the Makonde language and means "to become contorted", referring to the severe joint and muscle pains which occur in 70% to 99% of cases and result in a stooped posture. A rapidly rising high fever and further symptoms such as lymph node swelling, rash, punctual bleeding of the skin (petechia), bleeding of the nose or gums, headache, fatigue and inflammation of the eyes also occur.

Chikungunya fever subsides after around 10 days, generally without any lasting damage. In approximately 10% of patients the joint pains persist for more than three weeks or even months and years. In some cases complications such as accompanying hepatitis, meningitis, encephalitis or meningoencephalitis occur.

Dengue Viruses

DENV belong to the flavivirus family and comprise four different serotypes (DENV 1 to DENV 4). DENV are distributed in Latin America, Central Africa, India, South East Asia, in some parts of the Pacific islands and the Eastern Mediterranean. The virus is also regularly introduced to Europe. According to estimates of the WHO, there are around 50-100 million cases of dengue fever every year.

The disease initially manifests with flu-like symptoms such as high fever, severe headache, muscle and joint pains, exanthema and lymph node swelling. Severe complications in the form of dengue haemorrhagic fever or dengue shock syndrome occur in up to 1% of patients. The risk of haemorrhagic fever significantly increases after a second infection with a different DENV serotype. Severe dengue results in about half a million hospitalisations per year and around 20,000 deaths, many of them in children.

Acute Diagnostics

The most important laboratory methods for acute arboviral diagnostics are direct virus detection, serological tests and, for DENV, detection of the highly specific early antigen NS1. Direct detection by RT-PCR provides reliable identification of the infecting virus, but due to the short viraemic phase it is only effective within the first week after onset of symptoms in ZIKV, DENV and CHIKV infections (Figure 1). Thus, RT-PCR may already be negative by the time a patient consults a doctor. DENV NS1 antigen is detectable at the onset of clinical symptoms in both first and re-infections with DENV and remains detectable past the viraemic phase. NS1 detection serves as a first-line screening test for dengue and helps to minimise the diagnostic gap between RT-PCR and antibody positivity.

Serological methods are effective from soon after clinical onset to beyond convalescence. Antibodies against ZIKV, CHIKV and DENV appear around day four to seven after symptom onset. Acute infections are generally characterised by the occurrence of IgM, with IgG appearing at the same time or shortly thereafter. IgM antibodies reach their peak after two to three weeks and remain detectable for a few months, while IgG antibodies persist life-long. The detection of specific IgM antibodies or a significant rise in the specific IgG titer in a pair of samples taken seven to 10 days apart is evidence of an acute infection. Isolated positive IgG findings may indicate past contact with the virus. In secondary flavivirus infection, including DENV infection with a different serotype, IgM antibodies may be delayed, of reduced intensity or not detectable at all. In these cases a more than tenfold increase in the IgG concentration is observed.

Serological Applications

In addition to their application in acute diagnostics, serological methods are also useful for studying the long-term consequences of infection. For example, ZIKV serological investigations may help to establish if the dramatic rise in cases of microcephaly and Guillain-Barré syndrome observed in Zika-affected regions is a consequence of Zika virus infection. If the link to congenital malformations is confirmed, Zika virus serology could play an important future role in prenatal diagnostics. Pregnant women with serological evidence of an infection could be offered intense prenatal monitoring, while seronegative women may be spared unnecessary worry.

Serology is also useful for screening donated blood. Travellers returning from ▷

ZIKV, DENV and CHIKV endemic regions to non-affected countries should defer donation of blood for a few weeks or be confirmed as negative by PCR, depending on the respective organisation's recommendations. After this time, serological testing can verify the safety of donated blood products.

A further, critical role for serological studies is to monitor the growing epidemiological reach of arboviruses. As ZIKV, DENV and CHIKV are expected to continue to spread around the globe, knowledge about emerging endemic regions is valuable for providing effective patient diagnostics.

ELISA

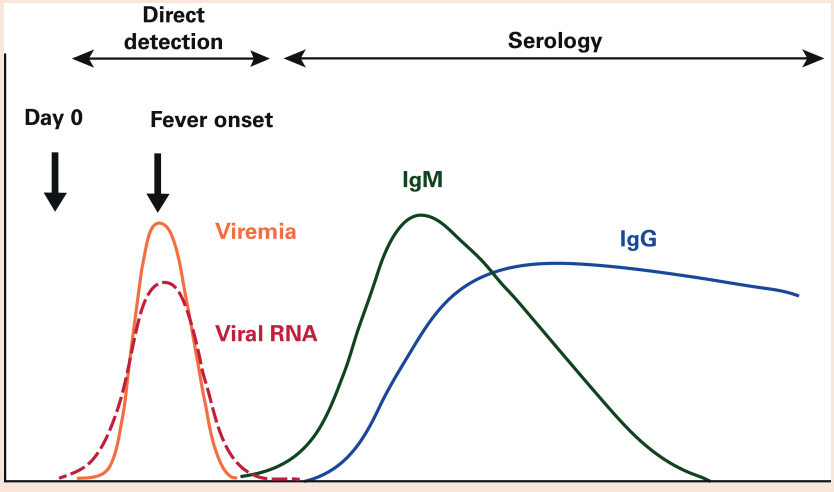
ELISAs provide fully automated measurement of antibody titers, and are a cost-effective method for screening large numbers of samples. They can be used to determine IgM or IgG antibodies.

Newly developed Anti-ZIKV ELISAs are based on a recombinant non-structural viral protein (NS1) from Zika virus. This highly specific antigen avoids cross-reactivity with other flaviviruses. In a first study with these tests, the IgM and IgG ELISAs showed 100% specificity in clinically and serologically characterised samples. Furthermore, the combination of IgM and IgG ELISAs ensures highest sensitivity of 97%. Data from well-characterised serum panels indicate that there is no cross-reactivity with flaviviruses like dengue, West Nile, yellow fever, tick-borne encephalitis and Japanese encephalitis viruses.

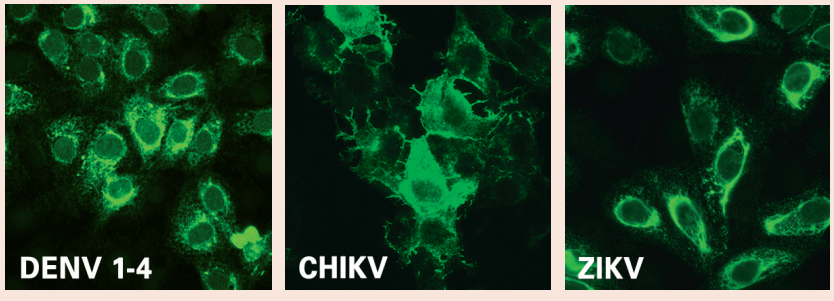
Anti-DENV ELISAs are based on highly purified virus particles of serotype 2. Due to the high structural similarity between DENV 1 to 4, use of one serotype is sufficient to detect antibodies against all four virus types. In clinically characterised sera the IgM and IgG ELISAs demonstrated 100% sensitivity and 99% specificity. They also showed very good correlation with other serological assays. Due to use of whole antigen, cross reactions with other flavivirus antibodies cannot, however, be excluded.

Anti-CHIKV ELISAs utilise a virus-specific structural protein as the antigenic substrate. In clinically characterised samples the IgM ELISA showed 100% sensitivity for detecting acute infections. In a published comparative study the Anti-CHIKV IgM and IgG ELISAs demonstrated the highest sensitivities of all diagnostic tests used, as well as high specificities. Further studies have confirmed the excellent overall agreement of the ELISAs with other serological assays.

▼FIGURE 1. Course of an arboviral



▼FIGURE 2. Positive IIFT reactions for DENV, CHIKV and ZIKV



Indirect Immunofluorescence

Antibody detection by IIFT is based on cells infected with the corresponding virus, which provide highly sensitive diagnostics. Positive and negative results are evaluated by fluorescence microscopy (Figure 2). In clinically characterised samples the IIFT substrates yielded sensitivities of 96% to 99% and specificities of 95% to 100% for the different parameters.

The Arboviral Fever Mosaic 2 consists of a combination of six substrates of cells infected with ZIKV, CHIKV and DENV serotypes 1 to 4, which are incubated in parallel. The mosaic can help in differential diagnostic delimitation of ZIKV, DENV and CHIKV infections. Due to the use of whole virus particles, cross-reactivity between antibodies against different flaviviruses, originating either from infections or vaccinations such as yellow fever, should be taken into account. Whilst there is generally no or only low-grade cross reactivity in a primary flavivirus infection, in a secondary flavivirus infection, for example a ZIKV infection following a DENV infection or vice versa, high-grade cross-reactivity is typical. The cross-reactivity is stronger for IgG than for IgM antibodies. Titration of the patient sample on this mosaic slide may enable determination of a dominant end-point titer for the virus causing the infection.

Perspectives

The dramatic global rise of ZIKV, DENV and CHIKV has placed billions of inhabitants and travellers at risk of infection. Moreover, it is anticipated that these febrile diseases will continue to spread into previously unaffected areas. Current strategies for managing outbreaks are focused on controlling local mosquito populations and avoiding personal exposure to bites. Vaccine research is also a priority. For persons who nevertheless become infected, efficient differential diagnostics by means of laboratory methods are indispensable. Molecular testing and serology form the backbone of diagnostic strategies. Serological analyses are, furthermore, crucial for monitoring the epidemiological reach and the long-term consequences of these diseases. The anti-ZIKV tests described here are the first commercially available assays for serological detection of anti-ZIKV antibodies. It is hoped that along with established anti-DENV and anti-CHIKV assays, they will help to combat these increasingly threatening diseases. ML