Commentary

Zika Virus Serology: More Diagnostic Targets, more Reliable Answers?

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Zika virus (ZIKV) is a mosquito-borne flavivirus and although it has been known for decades it largely remained obscured and cases were reported only sporadically (Weaver et al., 2016). In 2007, the situation changed dramatically upon the first large outbreak of ZIKV in the state of Yap, Federated States of Micronesia (Lanciotti et al., 2008). Subsequently, ZIKV spread to South America in 2015 causing explosive outbreaks throughout the continent.

In general, ZIKV infection remains asymptomatic or presents as a mild and short-lived febrile illness. Unexpectedly, however, an increase of cases of microcephaly was noticed in Brazil shortly after its emergence. There are now several lines of evidence supporting a causal relationship between ZIKV infection during pregnancy and congenital defects and malformations including microcephaly (Weaver et al., 2016). In addition, ZIKV infection can occasionally lead to Guillain-Barré syndrome, a post-infectious paralytic disease of the peripheral nervous system (Weaver et al., 2016).

ZIKV diagnosis is usually based on the detection of antibodies using ELISA since viremia is rather short-lived (Rabe et al., 2016). However, cross-reactive flavivirus antibodies can make the interpretation of serological results notoriously difficult. Plaque reduction neutralization test (PRNT) remains the gold standard to confirm and specify flavivirus antibodies. It works well in primary flavivirus infections but PRNT is labor-intensive, costly, and not widely available thus limiting its use for routine diagnostics. Serological diagnosis becomes more complex in individuals with previous flavivirus infections and/or vaccinations e.g. against yellow fever virus or tick-borne encephalitis virus. Even PRNT fails to establish a definite diagnosis in such cases which is of particular concern in areas where different flaviviruses are co-circulating. Public health laboratories and diagnostic companies have now developed novel assays to improve ZIKV diagnosis. Some of these assays received emergency use authorization by the US FDA, e.g. the Zika MAC-ELISA by Centers for Disease Control and Prevention or the Zika Detect IgM capture ELISA (InBios, Washington, USA).

In this issue of *EBioMedicine*, Wong et al. propose a microsphere immunoassay (MIA) using Luminex technology to diagnose ZIKV infection (Wong et al., 2017). This assay is based on a set of recombinant ZIKV structural (E protein) and nonstructural (NS1 and NS5) proteins. Recombinant Dengue virus (DENV) NS1 proteins were included to differentiate between ZIKV and DENV. Previous studies have shown that the E protein elicits flavivirus cross-reactive neutralization antibodies. On the contrary, the ZIKV NS1 protein induces a non-neutralizing yet virus-specific antibody response holding promise as a reliable diagnostic target (Stettler et al., 2016). In the study by Wong et al., proof-of-principal was provided using well characterized human sera with laboratory confirmed ZIKV and/or DENV infection and without markers of infection. The authors are to be commended to have uniformly used PRNT to pre-characterize their samples. In addition, they compared the novel MIA against a well-established ZIKV IgM-capture ELISA (MAC ELISA).

They could demonstrate that the novel MIA is equivalent or even more sensitive to detect ZIKV compared to MAC ELISA and that the immune response to ZIKV NS1/NS5 proteins is more virus-specific than to E protein. In particular in samples with primary PRNT confirmed flavivirus infection the sensitivity of the MIA ZIKV NS1 protein is 100% and compares to a sensitivity of 88% using MAC ELISA. Critically, the MIA detects IgM, IgA, and IgG antibodies limiting its use to differentiate acute from resolved infections. On the other hand, in samples with primary flavivirus infection the specificity of the MIA is 81% (ZIKV NS1 protein) and 94% (ZIKV NS5 protein), respectively, compared to 69% (MAC ELISA). This contrasts with findings of two recent studies using a ZIKV NS1 protein based assay which demonstrated a specificity of 96–100% in sera containing laboratory-confirmed antibodies against different flaviviruses including DENV (Huzly et al., 2016; Steinhagen et al., 2016). However, PRNT was not used in both studies. As expected for samples with secondary flavivirus infection, Wong et al. found a 22–36% cross reactivity between DENV and ZIKV NS1 proteins. Since only limited clinical information and no follow-up specimens were available to the authors it is difficult to interpret these data in more detail. In order to better appreciate the results of Wong et al. a direct comparison of the MIA to recently developed ZIKV NS1 protein based antibody assays seems prudent.

Technically, multiplexing offers the advantage to simultaneously test for different antibodies. In light of the co-circulation of flaviviruses in many ZIKV endemic regions the use of multiplex technology is a promising approach provided that the findings are further confirmed in future studies. Of note, the MIA needs to be run in a laboratory disposing of sophisticated machines limiting its use in resource limited regions. What is ultimately needed is a simple and easy to use field test.

Open research questions, which need to be addressed are the performance of the novel MIA using ZIKV seroconversion panels, sera of

residents from flavivirus endemic regions, and in particular sera from pregnant women. Further, as already discussed by the authors, an extension of the assay to detect antibodies against other arboviruses e. g. chikungunya virus would add value to the assay.

Considering these challenges future research should be directed on antibody kinetics and the extent of protection exerted by these antibodies. It is tempting to speculate that the level of ZIKV antibodies can be used as a correlate of protection (Larocca et al., 2016). Further, pre-existing flavivirus immunity may enhance ZIKV infection through antibody dependent enhancement (ADE) mediated by Fc-receptors in phagocytes (Dejnirattisai et al., 2016). However, ADE for ZIKV is controversial and epidemiological studies based upon evidence-based laboratory methods are needed. In sum, reliable diagnostic tools are instrumental to better appreciate the global burden of ZIKV and to achieve an effective public health response.

The author declares no conflicts of interest.

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