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Arbovirus Diagnostics: From Bad to Worse due to Expanding Dengue Virus Vaccination and Zika Virus Epidemics

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(See the Major Articles by Plennevaux et al on pages 1164–72 and Barzon et al on pages 1173–80.)

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Diagnosis of arthropod-borne viral infections (arboviruses) has been a challenge for decades as dengue viruses (DENVs) and other flaviviruses and alphaviruses have expanded their global reach. Serological detection of infection is complicated by the cross-reactivity of antibodies induced by these viruses, as well as the fact that frequent serial infections with related viruses provoke anamnestic recall responses to the primary infection. The recent introduction and use of DENV vaccines is likely to further complicate serological analysis of acute infection [1]. In this issue of *Clinical Infectious Diseases*, Plennevaux and colleagues examine the impact of DENV vaccination to all 4 DENV serotypes on subsequent serological detection of potential DENV infection [2]. Standard serological differentiation of acute DENV infection using immunoglobulin M (IgM) and immunoglobulin G (IgG) assays demonstrated high false-positive rates, particularly in vaccinated individuals. Also in this issue, Barzon and colleagues describe antibody responses following travel-acquired Zika virus (ZIKV) infection [3]. Of particular note, although ZIKV nonstructural protein 1 (NS1) IgM antibodies were detected at, or after, clinical symptom onset in cases without previous DENV antibodies, they were not detected in patients with previous dengue. These findings highlight the need for a closer examination of the limitations of current flavivirus diagnostics, particularly with regard to determining recently acquired infections in persons presenting with clinical syndromes (which overlap with many of these viruses) or in monitoring for recent (incident) infections in asymptomatic populations.

Dengue serology is complicated by 4 antigenically related serotypes, as well as other related flaviviruses, whereby cross-reactive serological responses may be stimulated by infection or vaccination [4]. Following DENV infection, seronegative individuals gain transient cross-reactive immunity [5], including detectable transient neutralization of other DENV serotypes [6]. This is exacerbated following secondary infection whereby persistent cross-reactive immunity is invoked [5] and at least transient neutralization of other flaviviruses, including ZIKV, can also be observed [7]. To further complicate matters, the antigenic similarities between the flaviviruses also lead to anamnestic responses to the historical primary flavivirus infections, or vaccination, which are restimulated by acute repeat flavivirus infection [4]. Anamnestic responses can occur at the expense of virus-specific responses to the current infection, in a phenomenon termed "original antigenic sin," making serological identification even more difficult. The recent explosive ZIKV epidemic in the Americas has brought this issue into sharp focus. Determining incident/recent ZIKV infection is of great importance in pregnant mothers, whose fetuses are at risk of congenital malformations and other syndromes linked to in utero or peripartum infection [8]. Co-circulation of DENV and other flaviviruses makes the issue of cross-reactivity substantial. Thus, at least 4 areas require further development in flavivirus serological tests and algorithms (optimally not requiring difficult and expensive confirmatory assays such as plaque reduction neutralization tests) to differentiate between (1) natural infection and vaccination (including current DENV vaccines and, in the near future, ZIKV vaccines); (2) serological responses to closely related flaviviruses, particularly DENV and ZIKV; (3) serological responses to the 4 DENV serotypes; and (4) recent and remote infection by each of these flavivirus.

In the current study by Plennevaux and colleagues, commercially available IgM and IgG enzyme-linked immunosorbent assays (ELISAs) based

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on inactivated DENV antigen were employed in vaccinated and control groups reporting febrile illness. As expected, serologically confirming recent DENV infection was effective in participants with febrile episodes with virologically confirmed DENV using either reverse-transcription polymerase chain reaction or NS1 antigen. However, in febrile participants without virologically confirmed DENV, a higher proportion of "probable dengue episodes" were described in the vaccinated group compared with the placebo controls, many of which were likely due to false-positive serological reactivity resulting from vaccine-induced responses. The majority of this difference was observed in those participants who were DENV naive (seronegative) at the time of entry into the study, with double the IgM false-positive rates in the vaccinated compared with the control groups.

Similarly, in the study by Barzon and colleagues, the dynamics of confirmed ZIKV cases in DENV-naive and -experienced individuals were followed over time. In flavivirus-naive patients, ZIKV NS1-specific IgM and IgG, measured with commercial assays, proved to be specific markers of recent ZIKV infection. However, in many of the DENV-experienced individuals, anti-NS1 IgG levels were already high at initial sampling, and did not demonstrate increases upon follow-up, suggesting significant cross-reactivity with existing DENV immunity. These studies confirm previous descriptions of cross-reactivity following ZIKV infection in flavivirus-experienced individuals, including difficulties differentiating between ZIKV-specific neutralizing antibody responses after acute infection with DENVs or ZIKV [9]. Furthermore, in the Barzon et al study, the DENV-experienced ZIKV patients failed to seroconvert anti-ZIKV NS1 IgM. Although this is consistent with IgM responses being lower in secondary DENV cases [10], current ZIKV serological assays using alternative antigens, such as whole virions [9] do not appear to have an issue with sensitivity in DENV-experienced individuals. Furthermore, next-generation assays based on ZIKV NS1, such as antigen sandwich assays [11] and blockage of binding using specific monoclonal antibodies to ZIKV NS1 [12, 13], demonstrate promising results in initial studies in terms of sensitivity in both DENV-naive and DENV-experienced situations. Thus, this may be an issue with sensitivity in the commercial assay used in the Barzon et al study [14]. Specific and sensitive anti-DENV NS1-based assays are also a likely long-term solution to the issue of differentiating natural infection from vaccine-induced immunity described by Plennevaux and colleagues as the CYD-TDV vaccine lacks DENV NS1. However, other vaccine candidates, such as TV003, are live attenuated viruses and thus express DENV NS1. Thus, other solutions will be required for differential serology to avoid detection of vaccine-induced seroreactivity and diagnose vaccine breakthrough infections.

In addition to the next-generation assays described above, other promising serological assays recently described for ZIKV include a plasmonic gold–based assay using either NS1 or ZIKV envelope proteins [15]. An alternative approach to highly specific novel assays is to use standard serological assays, such as ZIKV and DENV IgM and IgG ELISAs, and develop simple algorithms based on these assays and confirmatory assays for differentiating infection [16]. Initial studies for many of these assays and algorithms have been promising; however, their ability to fully differentiate ZIKV and DENV infection, as well as determine recent ZIKV infection, remains to be fully evaluated. To this end, in a study (https://reds-iii.rti.org/ResearchStudies/ZIKAStudies.aspx) funded by the National Heart, Lung, and Blood Institute, we have collected longitudinal samples up to 1 year post–index donations from asymptomatic blood donors identified as ZIKV infected using highly sensitive ZIKV nucleic acid amplification tests (NAATs) [17]. Many of these donors have high viral loads and are initially ZIKV IgM seronegative, allowing for characterization of evolving seroconversion, as well as development of symptoms and persistence of viral RNA and infectivity in various blood compartments and body fluids. The majority of these donor panels were collected in Puerto Rico during 2016, with some detected in continental US donors primarily with travel-acquired ZIKV, and thus are a mix of DENV IgG-seropositive and -negative at the time of index donations. Furthermore, we have similar panels of longitudinal samples from acutely DENV-infected donors from Puerto Rico detected by NAAT screening prior to 2015, and West Nile virus panels from the continental United States [18]. We are currently using these panels to assess the performance of several commercial and experimental serological assays for cross-reactivity and sensitivity over time periods consistent with acute or recent infection.

Although these recent advances in serological assay development are promising in the long term, there remains an immediate need to develop assays that can differentiate recent ZIKV infection from remote ZIKV infections, given that moderate proportions of persons living in South and Central America and the Caribbean islands are now seropositive for ZIKV antibodies as a result of large outbreaks in 2014–2016. The study by Barzon et al importantly confirms previous findings [19] concerning the potential usefulness of other sample types such as urine, saliva, and semen to prolong the window of viral RNA detection in acute patients. In their longitudinal studies, plasma viremia was only detectable for a median of 11 days. However, in 2 pregnant women enrolled in the study who likely were exposed before 12 weeks' gestation, plasma viremia in the women remained positive until delivery of apparently healthy infants, presumably reflecting ongoing ZIKV replication in the placenta or other reservoirs. This phenomenon has

been previously reported [20]; however, it is not apparent how common persistence of plasma viremia in pregnant women is, of if persistent RNA in plasma during pregnancies is prognostic for fetal infection or developmental abnormalities in the newborn. In contrast to plasma, the median time to clearance of RNA in urine was 24 days, also consistent with previous studies [19]. A caveat of these studies is that they were performed in symptomatic patients and there may be differences in the kinetics of RNA persistence in asymptomatically infected individuals. To address this, we have performed similar studies in acutely infected blood donors who are asymptomatic at the time of blood donation and are detected using highly sensitive ZIKV NAATs [17]. Our findings confirm extended detection of ZIKV RNA in urine and saliva for several weeks relative to clearance from plasma.

In addition to urine and saliva we, and others, have demonstrated that flavivirus infections (specifically West Nile virus and DENVs) lead to relatively long-term persistence of viral RNA in whole blood extracts and specifically erythrocyte (red blood cell [RBC]) concentrates [18]. Indeed, several studies of persons diagnosed with travel-acquired infections have demonstrated a similar phenomenon for ZIKV patients [21], and in our longitudinal studies of ZIKV-infected blood donors, persistence in whole blood and RBC samples can extend detection of ZIKV RNA to approximately 3 months postinfection (interestingly, the life span of RBCs).

At this point, a clinician who suspects ZIKV or DENV infection in a symptomatic case or is monitoring recent travelers, pregnant women, or their infants at risk for these infections should follow the latest Centers for Disease Control and Prevention diagnostic guidelines (https://www.cdc.gov/zika/index.html). These guidelines are revised and updated on a regular basis as new data and new assays become commercially available, including molecular tests with expanded emergency use authorization claims for testing of urine and blood. As we look to the future, promising studies suggest that new approaches to serological assays will allow for the differentiation of acute flavivirus infection from historical infection or vaccination despite the antigenic relatedness and high asymptomatic rate of DENV and other flaviviruses that complicates such analysis. Furthermore, analysis of viral RNA in different matrices such as whole blood and urine using highly sensitive blood donor screening NAATs will extend the viral RNA detection window to allow unequivocal diagnosis of a greater percentage of recent ZIKV infection in clinical cases and for monitoring pregnant women and persons who have traveled to outbreak regions.

Notes

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