Differential Shedding and Antibody Kinetics of Zika and Chikungunya Viruses, Brazil

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In seroconversion panels obtained from patients from Brazil, diagnostic testing for Zika virus infection was improved by combining multiple antibody isotypes, techniques, and antigens, but sensitivity remained suboptimal. In contrast, chikungunya virus diagnostic testing was unambiguous. Recurrent recent arbovirus infections suggested by serologic data and unpecific symptoms highlight the need for exhaustive virologic testing.

In 2013, Zika virus and chikungunya virus (CHIKV) emerged in Latin America (1,2). Their overlapping symptoms challenge accurate diagnosis on the basis of clinical manifestations (3). Direct Zika virus and CHIKV detection is limited to the acute phase of infection (4). Serologic detection of Zika virus–specific antibodies is hampered by low specificity and sensitivity of tests because of immune responses elicited by prior infection with other endemic flaviviruses (e.g., dengue virus [DENV]) (5,6). In addition, lack of adequate specimens limits studies evaluating the performance of diagnostic tests in tropical areas (7,8). To evaluate these challenges, we analyzed virus shedding and antibody responses over time in patients in Brazil sampled during the 2016 Zika virus and CHIKV outbreaks.

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The Study
We prospectively sampled patients in 4 time points up to 90 days post–symptom onset (dpo) (Table 1; Figure 1, panel A; Appendix, https://wwwnc.cdc.gov/EID/article/25/2/18-0166-App1.pdf). The cohort comprised 15 patients with acute Zika virus infection (5 male, 10 female; median age 39.0 years [interquartile range 31.0–44.0 years]) and 18 patients with acute CHIKV infection (10 male, 8 female; median age 39.0 years [interquartile range 31.0–57.3 years]), determined by detection of viral RNA in blood or urine 1–9 dpo (Appendix Figures 1, 2). All Zika virus belonged to the Asian lineage (2), and all CHIKV to the East/Central/South African lineage, according to envelope-based typing.

At enrollment, Zika virus patients most frequently reported fever, rash, and arthralgia (80% each), and CHIKV patients most frequently reported arthralgia (100%), fever (89%), and myalgia (89%) (Table 2). No co-infection with Zika virus, CHIKV, or DENV was detected by real-time reverse transcription PCR (rRT-PCR). However, serologic analyses found that 4 (27%) Zika virus–infected patients also had CHIKV IgM at enrollment, and 1 (7%) had DENV IgM (Appendix Table 1, Figure 3). Similarly, 3 (17%) CHIKV-infected patients had Zika virus IgM, and 4 (22%) CHIKV-infected patients had DENV IgM at enrollment (Appendix Figure 4). We cannot exclude the possibility of cross-reactivity between Zika virus–specific and DENV-specific antibodies because 2 CHIKV patients simultaneously showed Zika virus and DENV IgM in an envelope-based ELISA (Appendix Table 2). Seventy-nine percent of Zika virus and 83% of CHIKV patients showed serologic evidence for past DENV infection at enrollment (Appendix Figures 1, 2). Thus, recent infections with heterologous arboviruses might bias attributing infection-specific symptoms for Zika virus and CHIKV.

Consistent with previous studies (4,9), Zika virus loads in serum and urine were low up to 9 dpo (≈10^4 RNA copies/mL) (Figure 1, panel B), whereas CHIKV loads were ≈100-fold higher (≈10^6 RNA copies/mL) (Figure 1, panel C). However, unlike with Zika virus, CHIKV loads decreased significantly (p<0.001 by t test) from 5 dpo onward, and viral loads in urine were consistently low (Figure 1, panels D, E).

Next, to assess the antibody kinetics of Zika virus and CHIKV, we measured antibody responses over time.
logic methods as an alternative or additional marker to
This finding supports the usability of IgA-based sero-
convalescence (Figure 2, panel B; Appendix Figure 3).
and subacute phases of infection and decreased during
patients also showed IgA, which increased during acute
infection increased the detection rate to 53% (8/15) over
Table 1
gest comparable specificity of IgM detection (Appendix
sensitivity, concordant results from different assays sug-
DQ210
DQ195
DQ170
DQ144
DQ141
DQ138
DQ135
DQ132
DQ131
DQ128
DQ125
DQ122
DQ119
DQ116
DQ113
DQ110
DQ87
DQ84
DQ81
DQ78
DQ75
DQ72
DQ70
DQ67
DQ64
DQ61
DQ58
DQ55
DQ52
DQ49
DQ46
DQ43
DQ40
DQ37
DQ34
DQ31
DQ28
DQ25
DQ22
DQ19
DQ16
DQ13
DQ10
DQ7
DQ4
DQ1

Table 1. Sampling details for retrospective study of differential
shedding and antibody kinetics of Zika virus and CHIKV,
Brazil, 2016*

<table>
<thead>
<tr>
<th>Sample no.</th>
<th>Virus detected</th>
<th>Days from symptom onset to sampling</th>
<th>Collection date of acute-phase samples</th>
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<tbody>
<tr>
<td>DQ005</td>
<td>Zika virus</td>
<td>2</td>
<td>Mar 14</td>
</tr>
<tr>
<td>DQ028</td>
<td>Zika virus</td>
<td>1</td>
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</tr>
<tr>
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<td>3</td>
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<td>DQ047</td>
<td>Zika virus</td>
<td>2</td>
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</tr>
<tr>
<td>DQ049</td>
<td>Zika virus</td>
<td>1</td>
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<tr>
<td>DQ058</td>
<td>Zika virus</td>
<td>4</td>
<td>Mar 30</td>
</tr>
<tr>
<td>DQ060</td>
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</tr>
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<td>DQ075</td>
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<td>DQ108</td>
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<td>Apr 18</td>
</tr>
<tr>
<td>DQ246</td>
<td>Zika virus</td>
<td>5</td>
<td>Jun 24</td>
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<tr>
<td>DQ030</td>
<td>CHIKV</td>
<td>3</td>
<td>Mar 21</td>
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<td>Mar 30</td>
</tr>
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<td>CHIKV</td>
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<tr>
<td>DQ074</td>
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<td>1</td>
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<td>Apr 6</td>
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<td>Apr 7</td>
</tr>
<tr>
<td>DQ097</td>
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<td>3</td>
<td>Apr 11</td>
</tr>
<tr>
<td>DQ113</td>
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<td>5</td>
<td>Apr 13</td>
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<tr>
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<td>4</td>
<td>Apr 25</td>
</tr>
<tr>
<td>DQ170</td>
<td>CHIKV</td>
<td>2</td>
<td>May 3</td>
</tr>
<tr>
<td>DQ195</td>
<td>CHIKV</td>
<td>2</td>
<td>May 11</td>
</tr>
<tr>
<td>DQ210</td>
<td>CHIKV</td>
<td>2</td>
<td>May 16</td>
</tr>
<tr>
<td>DQ220</td>
<td>CHIKV</td>
<td>4</td>
<td>May 17</td>
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*CHIKV, chikungunya virus.

by commercial and in-house serologic tests. In a widely
used nonstructural (NS) protein 1 antigen-based ELISA,
Zika virus IgM seroconversion was low (33% [5/15]),
whereas CHIKV IgM seroconversion was 100% using an
everwarp-based ELISA (p<0.0001 by Fisher exact
test) (Figure 2, panel A; Appendix Tables 1, 2). Use of an
in-house envelope-based ELISA increased the Zika virus
IgM detection rate to 50% (7/14), and use of a commercially available μ-capture ELISA increased it to
43% (6/14) (Figure 2, panel A). Despite differential
sensitivity, concordant results from different assays sug-
gest comparable specificity of IgM detection (Appendix
Table 1). The use of NS1-based IgA as a marker of acute
infection increased the detection rate to 53% (8/15) over
that of the NS1-based IgM ELISA. All IgM-positive pa-
tients also showed IgA, which increased during acute and subacute phases of infection and decreased during
convalescence (Figure 2, panel B; Appendix Figure 3).
This finding supports the usability of IgA-based sero-
logic methods as an alternative or additional marker to
IgM-based methods to detect acute Zika virus infection.
The detection rate increased 2-fold when we used NS1-
based IgA from when we used NS1-based IgM 5–9 dpo,
suggesting that IgA could be used at later stages of in-
fected (Appendix Figures 1, 5). Our findings indicate
that serologic detection of acute Zika virus infection can
be improved s2-fold by use of different antibody classes
and antigens but remains poorly sensitive in flavivirus-
endemic areas.

All Zika virus–infected patients showed IgG responses
across the 4 time points in ≥1 assay (Figure 2, panels C,
D). Plaque reduction neutralization tests (PRNTs) were
negative for 2 of 14 rRT-PCR–confirmed Zika virus cases
detected by NS1-based IgG ELISA. Without rRT-PCR
confirmation, these cases would have been classified false
positive (Appendix Table 1). This observation might be
explained by differential sensitivity of PRNT and ELISA
(10) or false-positive results of the Zika virus NS1-based
ELISA in secondary flavivirus infections (6). Similarly, the
antibody kinetics of Zika virus NS1-based IgG, envelope-
based IgG, and PRNT suggested either relatively early
IgG seroconversion or cross-reactivity during acute stages
of infection resulting from unspecific immune responses
against other flaviviruses (11) (Figure 2, panel D). In con-
trast, CHIKV IgG seroconversion occurred at later stages
(Figure 2, panel D; Appendix Figure 5), possibly associ-
ated with strong and long-lasting CHIKV-specific IgM re-
sponses (Appendix Figure 4).

Conclusions
We provide pivotal data on Zika virus and CHIKV diag-
nostic challenges in a Latin American setting. Limitations
of our study include the relatively small number of pa-
tients, sampling at heterogeneous dpo and heterogeneous
numbers of samples per dpo, and lack of acutely DENV-
infected patients to assess test specificity. The strengths of
our study include rRT-PCR–confirmed infections, waiv-
ing the need to define serologic assays prone to cross-
reactivity as standards, sampling during Zika virus and
CHIKV outbreaks (1,2), sequential sampling of patients
up to 90 dpo, use of multiple antigens and immunoglobu-
lin classes, and the combination of molecular and sero-
logic testing methods.

Our data suggest reliable diagnostic testing for acute
CHIKV infections by IgM detection from 5 dpo onward.
This finding might enable waiving labor-intensive and costly
molecular protocols in many patients, minimizing costs for
public health systems and cohort studies investigating ar-
bovirus pathogenesis. However, reliability of CHIKV sero-
logic diagnostic tests must be reevaluated for co-circulating
genotypes (12) and for the antigenically related Mayaro vi-
rus (13) if it emerges in Latin America.

The difficulties of adequately diagnosing Zika vi-
rus infections in areas to which it is endemic have major
implications for public health. Reliable testing for flaviviruses in such areas will be key for epidemiologic studies on Zika virus and assessments of the safety of flavivirus vaccination programs, as illustrated by more severe dengue infections in DENV-seronegative individuals who received a live attenuated dengue vaccine (14).

For pregnant women and couples intending pregnancy, accurate diagnosis of acute or past Zika virus infection is crucial. The steep increase in requests for abortion in Latin America illustrates the effect of the Zika virus outbreak on reproductive medicine (15).

Our results highlight that definite exclusion of acute Zika virus infections is challenging in a considerable proportion of patients. However, although limited by a small number of samples, our data highlight the attainability of more accurate Zika virus diagnostic testing by combining molecular and serologic tests using different antibody classes, antigens, and methods and by monitoring an increase of IgG titers in follow-up serum samples. Our data will help clinicians and health authorities build reliable diagnostic algorithms for Zika virus and CHIKV and highlight that exhaustive testing of arboviral infections is required for attributing frequencies of infection-specific symptoms.

Acknowledgments

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Table 2. Symptoms of Zika virus and CHIKV reported by patients at enrollment 1–5 days after symptom onset, Brazil, 2016*

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Zika virus, no. (%), n = 15</th>
<th>CHIKV, no. (%), n = 18</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rash</td>
<td>12 (80)</td>
<td>9 (50)</td>
</tr>
<tr>
<td>Fever</td>
<td>12 (80)</td>
<td>16 (89)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>12 (80)</td>
<td>18 (100)</td>
</tr>
<tr>
<td>Myalgia</td>
<td>9 (60)</td>
<td>16 (89)</td>
</tr>
<tr>
<td>Cephalgia</td>
<td>8 (53)</td>
<td>12 (67)</td>
</tr>
<tr>
<td>Retro-orbital pain</td>
<td>5 (33)</td>
<td>8 (44)</td>
</tr>
<tr>
<td>Edema</td>
<td>4 (27)</td>
<td>3 (17)</td>
</tr>
<tr>
<td>Nausea, vomiting</td>
<td>3 (20)</td>
<td>6 (33)</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>2 (13)</td>
<td>5 (28)</td>
</tr>
</tbody>
</table>

*CHIKV, chikungunya virus.
About the Author
Dr. F.A. Bozza is a senior scientist and head of the Laboratório de Medicina Intensiva, Fundação Oswaldo Cruz Rio de Janeiro. His research focuses on the host immune response and metabolic adaptation to severe infections.

References

Figure 2. Zika virus and CHIKV antibody dynamics among samples from patients in Brazil, 2016. A) Percentage seroconversion for markers of acute infection with Zika virus and CHIKV (IgM NS1-based Zika virus ELISA, IgM envelope-based Zika virus ELISA, IgM µ-capture Zika virus ELISA, IgA NS1-based Zika virus ELISA, IgM CHIKV ELISA) at any time point. B) Median ELISA ratios for Zika virus and CHIKV IgM and IgA over time. C) Percentage seroconversion for markers of convalescence after Zika virus and CHIKV infection (IgG NS1-based Zika virus ELISA and IgG envelope-based Zika virus ELISA, Zika virus PRNT<sub>50</sub>, IgG CHIKV ELISA) at any time point. D) Median ELISA ratios for Zika virus and CHIKV IgG over time. Numbers of specimens per time point are shown in Figure 1. Dashed lines indicate signal-to-cutoff ratios of ≥1.1 considered positive for all ELISAs except µ-capture ELISA, for which the dashed line indicates a signal-to-cutoff ratio of ≥10, considered positive by the manufacturer. See Appendix Figure 5 (https://wwwnc.cdc.gov/EID/article/25/2/18-0166-App1.pdf) for the percentage de novo seroconversion of Zika virus and CHIKV in different assays per time point. CHIKV, chikungunya virus; dpo, days post–symptom onset; E, envelope; NS, nonstructural protein; PRNT, plaque reduction neutralization test.


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