Title

Maternal Zika Virus Disease Severity, Virus Load, Prior Dengue Antibodies, and Their Relationship to Birth Outcomes.

Journal

Clinical Infectious Diseases, 65(6)

ISSN

1058-4838

Authors

Halai, Umme-Aiman
Nielsen-Saines, Karin
Moreira, Maria Lopes
et al.

Publication Date

2017-09-15

DOI

10.1093/cid/cix472

Peer reviewed

Clinical Infectious Diseases

MAJOR ARTICLE

Maternal Zika Virus Disease Severity, Virus Load, Prior Dengue Antibodies, and Their Relationship to Birth Outcomes

Umme-Aiman Halai,[1] Karin Nielsen-Saines,[1] Maria Lopes Moreira,[2] Patricia Carvalho de Sequeira,[2] Jose Paulo Pereira Junior,[2] Andrea de Araujo Zin,[2] James Cherry,[1] Claudia Raja Gabaglia,[3] Stephanie L. Gaw,[4] Kristina Adachi,[1] Irena Tsui,[1] Jose Henrique Pilotto,[2] Rita Ribeiro Nogueira,[2] Ana Maria Bispo de Filippis,[1] Patricia Brasil[2]

[1]David Geffen School of Medicine, University of California, Los Angeles; [2]Fundação Oswaldo Cruz, Rio de Janeiro, Brazil; [3]Biomedical Research Institute of Southern California, Oceanside; [4]School of Medicine, University of California, San Francisco.

Background. Congenital Zika virus (ZIKV) syndrome is a newly identified condition resulting from infection during pregnancy. We analyzed outcome data from a mother-infant cohort in Rio de Janeiro in order to assess whether clinical severity of maternal ZIKV infection was associated with maternal virus load, prior dengue antibodies, or abnormal pregnancy/infant outcomes.

Methods. A clinical severity assessment tool was developed based on duration of fever, severity of rash, multisystem involvement, and duration of symptoms during ZIKV infection. ZIKV-RNA load was quantified by polymerase chain reaction (PCR) cycles in blood/ urine. Dengue immunoglobulin G (IgG) antibodies were measured at baseline. Adverse outcomes were defined as fetal loss or a live infant with grossly abnormal clinical or brain imaging findings. Regression models were used to study potential associations.

Results. 131 ZIKV-PCR positive pregnant women were scored for clinical disease severity, 6 (4.6%) had mild disease, 98 (74.8%) had moderate disease, and 27 (20.6%) severe manifestations of ZIKV infection. There were 58 (46.4%) abnormal outcomes with 9 fetal losses (7.2%) in 125 pregnancies. No associations were found between: disease severity and abnormal outcomes ($P = .961$; odds ratio [OR]: 1.00; 95% confidence interval [CI]: 0.796–1.270); disease severity and viral load ($P = .994$); viral load and adverse outcomes ($P = .667$; OR: 1.02; 95% CI: 0.922–1.135); or existence of prior dengue antibodies (88% subjects) with severity score, ZIKV-RNA load or adverse outcomes ($P = .667$; OR: 0.78; 95% CI: 0.255–2.397).

Conclusions. Congenital ZIKV syndrome does not appear to be associated with maternal disease severity, ZIKV-RNA load at time of infection or existence of prior dengue antibodies.

Keywords. Zika; dengue; ZIKV; pregnancy; congenital.

Zika virus (ZIKV) infection in adults has been described as a dengue-like viral illness, characterized by low-grade fever, a pruritic macular or maculo-papular rash, headache, conjunctival hyperemia, myalgia and arthralgia, usually lasting between 5 to 10 days [1–6]. Due to the potential for cross-reactivity with other flaviviruses, particularly dengue, it is difficult to confirm ZIKV-specific antibodies in readily available serological tests. Diagnosis relies on detection of ZIKV by polymerase chain reaction (PCR) in serum or urine during the period of acute ZIKV infection, which has been estimated in most cases to be ranging between 0–14 days in serum and 0–21 days in urine [3–7]. In the absence of a reliable and reproducible serological test, asymptomatic or mild clinical infection that is presumed to occur in 80% of cases [1], cannot be confirmed outside this window. Therefore, ZIKV infection is a diagnostic challenge in sites where dengue infection is endemic. In addition, despite its benign presentation in most adults, ZIKV can be extremely pathogenic to the developing fetus. Causality between ZIKV infection during pregnancy and fetal neurological abnormalities has been clearly established, and microcephaly appears to be only the tip of the iceberg [8–10]. It has not been determined whether there is an association between clinical disease due to ZIKV infection, virus load or pregnancy, and infant outcomes.

Our team recently published findings from a prospective cohort of 345 pregnant women in Rio de Janeiro, Brazil, where microcephaly was observed in 4 of 126 infants born to ZIKV-PCR positive pregnant women (3.2%) [10]. Overall, our rate of abnormal pregnancy outcomes was as high as 46%. Among 126 infants, 42% had abnormalities consistent with congenital ZIKV infection and occurred regardless of the trimester of maternal ZIVK infection. Following publication of this high frequency of abnormalities, questions arose as to whether symptomatic ZIKV infection may more frequently lead to adverse pregnancy and infant outcomes. Other studies have reported lower rates

Received 7 February 2017; editorial decision 10 May 2017; accepted 17 May 2017; published online May 23, 2017.

Correspondence: U.-A. Halai, Department of Medicine, David Geffen UCLA School of Medicine, CHS 37–121, LeConte Ave, Los Angeles, CA 90049 (aiman.shabbir@gmail.com).

Clinical Infectious Diseases® 2017;65(6):877–83

DOI: 10.1093/cid/cix472

of infant abnormalities when evaluating infants born to women with either symptomatic or asymptomatic ZIKV infection [11, 12]. It also has been hypothesized that infants born to women in endemic areas may have a higher rate of abnormalities due to the presence of dengue antibody dependent enhancement phenomena [13, 14]. For these reasons we performed an analysis using our ZIKV pregnancy cohort data exploring potential associations between clinical severity of maternal ZIKV infection, virus load at the time of acute infection, presence of dengue antibodies, and pregnancy/infant outcomes.

METHODS

This analysis was conducted in a prospective cohort of pregnant women with new-onset rash referred to the Acute Febrile Illness Branch of the Oswaldo Cruz Foundation (Fiocruz), Rio de Janeiro. The study enrolled 345 pregnant women as of September 2015 with 53% testing positive for ZIKV by real time PCR on serum and/or urine specimens. The current report focuses on 134 ZIKV-PCR positive women enrolled in our cohort until May 2016, for whom 125 pregnancy outcomes were known by July 31, 2016. Details of recruitment, enrollment, and follow-up of these patients have been previously published [10].

Clinical Severity Assessment

The clinical severity scoring criteria was established *a priori* based on literature review and local experience following a consensus meeting among our investigators prior to conducting the present analysis. The clinical presentation of disease was scored in 4 categories as detailed in Table 1. These included: (1) severity of rash, (2) duration of fever, (3) multisystem involvement, and (4) duration of illness. Each category was given a minimum score of 1 for mild and a maximum of 3 for severe. The severity of presentation was calculated as a sum of the 4 individual categories, with a minimum of 4 and maximum of 12. The overall clinical severity score was divided into 3 severity grades and categorized as mild, moderate or severe based on total scores of 4 or less, 5–8, and 9–12, respectively.

Viral Load Assessment

The Lanciotti real-time reverse transcription PCR (RT-PCR) methodology was used for ZIKV RNA detection [15], with results expressed as amplification cycles of PCR or cycle times (CT) at which ZIKV RNA was detected. Lower threshold cycles indicate a higher RNA burden. As both urine and serum samples were tested, when both specimens were positive, the lower CT-values (i.e., indicative of a higher viral load) were used for overall analysis. As it is not certain that RNA loads from different body compartments are comparable [7], we also conducted subgroup analysis by type of specimen (serum or urine).

Birth Outcomes

Birth outcomes were divided broadly into normal and abnormal based on study findings. Normal outcome was defined as a live birth of an infant with no apparent abnormalities on physical exam and neonatal imaging studies. Abnormal findings included pregnancy loss, abnormal infant physical exam, or abnormal infant neuro-imaging findings. The outcomes were further subdivided into the following groups: (1) normal, (2) abnormality on clinical neurological assessment only, (3) structural or imaging abnormality with or without abnormality on clinical exam, and (4) fetal loss.

Table 1. Clinical Severity Scoring Criteria

Category	Description	Grade	Score
Severity of rash	Rash was graded based on extent or intensity	Mild: 1+	1
		Moderate: 2+	2
		Severe: 3+	3
Duration of fever		Mild: No fever	1
		Moderate: Fever lasting 1–2 days	2
		Severe: Fever lasting ≥ 3 days	3
Multisystem involvement	One point was given for each of the following	Mild: ≤ 2	1
	Fatigue or lightheadedness	Moderate: 3–4	2
	Arthralgia or arthritis or peri-articular edema	Severe: ≥ 5	3
	Myalgia		
	Headache or photophobia or retro-orbital pain or tremor or paresthesia		
	Anorexia or nausea/vomiting or abdominal pain or diarrhea		
	Lymphadenopathy		
Duration of illness	From the start of first symptom to the end of any symptom	Mild: ≤ 5 days	1
		Moderate: 6–9 days	2
		Severe: ≥ 10 days	3

Clinical Severity Score: Sum of each category above (range 4–12).

Clinical Severity Grade: Mild score = 4, Moderate score = 5–8, Severe score = 9–12.

Statistical Analysis

Medians, interquartile ranges, ranges, and percentages were used to describe data. Box plots and bar graphs were used to visualize findings. We used logistic and linear regression models to study associations between clinical severity scores, CT values, and pregnancy outcomes. We used maternal age, gestational age at infection, and day of illness at evaluation from symptom onset in models to control for confounding. All analysis was conducted using STATA statistical software (StataCorp, LP, College Station, Texas, USA).

RESULTS

Baseline clinical characteristics are detailed in Table 2. Sixteen patients were assessed >7 days from the onset of symptoms; however, all women were assessed ≤5 days from the onset of rash. Among 134 ZIKV-PCR positive pregnant women, 131 had detailed prenatal data available for symptom scoring. Three patients were initially assessed at private facilities, and detailed symptom evaluation at time of infection was not available for scoring. As seen in Figure 1, based on clinical severity grade assessment, 6 patients met criteria for mild clinical disease (4.6%), 98 patients met criteria for moderate clinical disease (74.8%), and 27 patients met criteria for severe disease (20.6%). The range of scores was 4–11; with a median score of 7 and an interquartile range (IQR) of 6–8.

Table 2. Baseline Clinical Characteristics of ZIKV PCR Positive Pregnant Women

Median Age (years)	31 (Range 16–46)
Median gestational age at infection (weeks)	24 (range 6–39)
First trimester infections	16/134 (11.9%)
Second trimester infections	74/134 (52.2%)
Third trimester infections	44/134 (32.8%)
Signs and symptoms	
Fever	34/124 (27.4%)
Fever lasting > 2 days	4/124 (3.2%)
Rash	
Mild	31/131 (23.6%)
Moderate	65/131 (49.6%)
Severe	35/131 (26.7%)
Neurologic symptoms (Headache or photophobia or retro-orbital pain or tremor or paresthesia)	92/131 (70.2%)
Gastrointestinal symptoms (Anorexia or nausea/vomiting or abdominal pain or diarrhea)	70/131(53.4%)
Fatigue or lightheadedness	68/131 (58.2%)
Myalgia	53/130 (36.9%)
Arthralgia or arthritis or peri-articular edema	81/130 (62.3%)
Lymphadenopathy	48/125 (38.4%)
Edema	54/99 (54.5%)
Conjunctival injection	73/127 (57.5%)
Multisystem involvement (other than fever and rash)	
1–2 systems	47/134 (35.1%)
3–4 systems	53/134 (39.6%)
> 5 systems	34/134 (25.45)
Median duration of symptomatic illness	7 (range 3–68)
Duration ≤ 5 days	43 (32.8%)
Duration 6–9 days	60 (45.8%)
Duration ≥ 10 days	28 (21.4%)
Median day of symptoms at evaluation	4 (range 1–31)

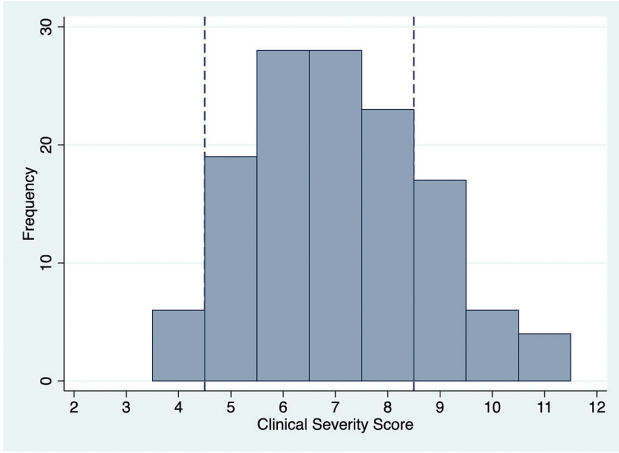

Figure 1. Distribution of Zika virus infection clinical severity scores. Reference lines indicate severity grades; mild presentation score = 4, moderate presentation score = 5–8, severe presentation score = 9–12.

For 134 ZIKV-PCR positive pregnant women, 130 PCR results were done at our facility with quantitative results available for analysis. Of these, 31 (24%) were positive specimens in both urine and serum, 45 (35%) were positive in urine only, and 54 (42%) were positive in serum only, for a total of 85 positive PCR results in serum and 76 positive results in urine specimens. The median number of CT values for serum specimens was 32 (IQR, 30 to 34; range, 24 to 37). The median number of CT values for urine specimens was 30 (IQR, 27 to 33; range, 22 to 37). As detailed in Figure 2a, for mild severity of clinical presentation, the median ZIKV CT-values in serum or urine was 30 (IQR, 28–32; range, 28–35); for moderate severity, the median CT-values in serum or urine was 31 (IQR, 28–33; range, 22–37) and for severe clinical disease presentation, the median number of ZIKV-PCR CT-values on serum or urine was 31 (IQR, 28–34; range, 24–37). In our linear regression analysis, we found no association between clinical severity score and viral RNA load expressed as amplification cycles of ZIKV PCR (P = .994) after controlling for age, gestational age at infection, and days from symptom onset. This relationship remained non-significant even on subgroup analysis by specimen type; serum (P = .644) and urine (P = .890).

Dengue immunoglobulin G (IgG) serology (Abcam, Cambridge, Massachusetts, USA), obtained at the time of acute presentation of ZIKV infection was available for 121 of 134 ZIKV-PCR positive pregnant women; 107 women were IgG positive (88.4%). Among 125 women with known pregnancy and infant outcomes, dengue IgG was available for 112 subjects with 98 women (87.5%) having positive IgG results and 14 (12.5%) having negative results. For women without evidence of previous dengue infection, the median clinical

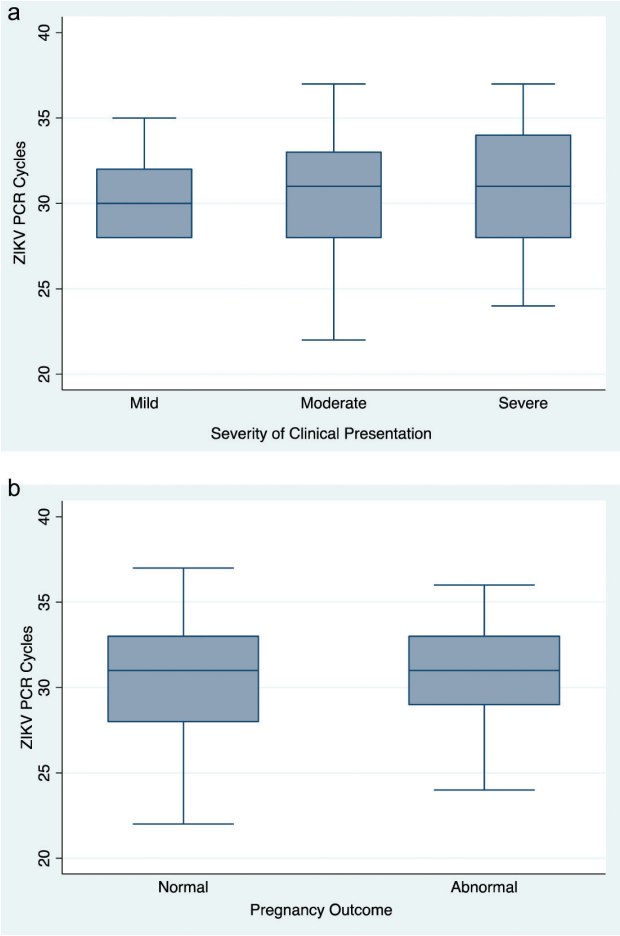

Figure 2. *A,* ZIKV PCR cycles by severity grade of clinical presentation. Abbreviation: *B,* ZIKV PCR cycles according to pregnancy outcomes. Abbreviation: PCR, polymerase chain reaction.

severity score was 7.5 (range: 4–11; IQR 6–9). For women with evidence of previous dengue infection, that is, IgG positive, the median clinical severity score was 7 (range: 4–11; IQR 6–8). Among dengue IgG positive women, the median CT-value for ZIKV detection was 31 (range: 22–37; IQR 28–33). For dengue IgG negative subjects, the median CT value was 32 (range: 26–37; IQR 30–34). There was no statistically significant effect of pre-existing dengue antibodies on clinical severity of ZIKV infection ($P = .166$) or ZIKV RNA load by CT values ($P = .153$) in our cohort. Seven of 14 (50%) dengue IgG negative, ZIKV-PCR positive pregnant women had abnormal birth outcomes, and 43 of 98 (43.8%) dengue IgG positive, ZIKV-PCR positive pregnant women had abnormal birth outcomes. We detected no effect of pre-existing dengue antibodies on abnormal birth outcomes in ZIKV infected pregnant women ($P = .667$; OR 0.78; 95% CI: 0.255–2.397).

In our cohort of 134 ZIKA PCR positive pregnant patients, 125 birth outcomes were available [10]. Fifty-eight (46.4%) pregnancies were found to have abnormal outcomes, including 9 fetal losses (7.2%). In sum, 117 live birth infants occurred in 116 women (one set of twins). Forty-nine infants (42%) were found to have abnormal outcomes. These outcomes have been previously described [10]. Thirty-seven (29.4%) ZIKV-exposed infants had structural or imaging abnormalities, and an additional 12 babies (9.5%) had abnormal clinical neurological assessments in the first months of life. As detailed in Figure 2b, pregnancy and infant outcomes observed in our cohort were not associated with maternal viral load as measured by CT values. ($P = .666$; OR 1.02; 95% CI: 0.922–1.135). This remained nonsignificant when viral PCR was stratified according to type of specimen (serum or urine). In addition, as seen in Figure 3, an association between clinical presentation severity score and probability of an abnormal outcome (including fetal demise or structural, clinical, or imaging abnormalities confirmed after birth) was not demonstrated in our analysis ($P = .961$; OR 1.00 95% CI: 0.796 –1.270). This association remained statistically nonsignificant even after outcomes were subdivided into fetal loss, structural/imaging abnormality with or without clinical neurological impairment and abnormality by clinical neurological assessment only (Table 3).

As seen in Table 4, the only parameter found to have an association with pregnancy outcomes in the multivariable analysis was gestational age at infection. There was a protective effect of infection in later gestational time points ($P = .042$, OR 0.95, 95% CI: 0.911–0.998). The odds of an abnormal pregnancy outcome decreased by 5% for every additional week of gestation prior to infection.

DISCUSSSION

We are only beginning to understand the pathogenesis and impact of ZIKV infection in pregnancy. Although most studies have shown the greatest risk in first trimester infection, adverse outcomes including fetal loss have been reported across all trimesters [9, 10, 16, 17]. Pregnancy is considered to be an

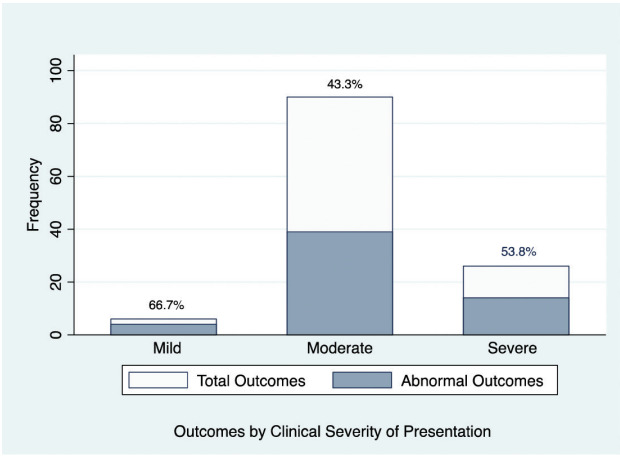

Figure 3. Pregnancy and infant outcomes according to maternal Zika virus disease severity.

Table 3. Pregnancy Outcomes According to Clinical Presentation Severity of Prenatal ZIKV Infection

Clinical Severity Grade	Pregnancy Outcome[a]			
	Normal	Clinical Neurological Assessment Abnormality Only	Structural Imaging Abnormality +/- Clinical Neurological Impairment	Fetal Loss
Mild	2	0	4	0
Moderate	50	7	24	9
Severe	13	4	9	0
Total (%)	65 (53.3)	11 (9.0)	37 (30.3)	9 (7.4)

[a]Out of 125 completed pregnancies with known outcomes, 122 had both outcome and clinical severity scores shown above.

immune-altered state as the immune system of the pregnant woman adapts to tolerate the foreign fetus [18–20]. Due to the immunological changes in pregnancy, other infections have more severe manifestations during gestation, as is the case of measles, influenza, coccidiodomycosis, or hepatitis E. To what extent the altered immune status due to pregnancy impacts the clinical presentation and pathogenicity of ZIKV is unknown at this time.

For many acute infections in immunocompetent adults, one expects the severity of signs and symptoms to correspond to the level of bacteria, toxins, or virus circulating in the body. However, no correlation was found between clinical disease severity and ZIKV-RNA load detected by PCR in our cohort of ZIKV-infected pregnant women. Studies on HIV, hepatitis B and C in pregnancy have shown a greater risk of in utero transmission to the fetus at higher viral loads [21–23]. This is an important consideration when planning treatment and prevention of mother to child transmission of these infections. In our analysis, we did not observe increased risk of congenital ZIKV syndrome in infants whose mothers had higher RNA loads in either serum or urine. It is possible that, like Parvovirus infection, viremia and infectivity is maximal before the onset of rash [24] and because all of our patients were referred after the onset of a rash, the peak viral load could not be detected. However, 88% of the specimens were collected within a week of any reported symptom, and even if the PCR cycle times do not represent maximum viral load, they represent magnitude of viremia/viruria at approximately the same time from symptom onset. Thus, differential assessment can still be undertaken with regard to correlation with clinical presentation or pregnancy outcomes. As longitudinal assessments to demonstrate duration of viral shedding were not performed, an association with duration of viremia cannot be ruled out.

Table 4. Multivariable Logistic Regression Showing Odds Ratio of Abnormal Outcome[a]

	Odds Ratio	*P* Value	95% Confidence Interval
Clinical severity score	1.00	.961	0.796 –1.270
Zika-polymerase chain reaction cycle times (urine or serum)	1.02	.666	0.922 –1.135
Age (y)	1.05	.114	0.988–1.11
Gestational age at infection (wk)	0.95	.042	0.911–0.998
Time from first reported symptom to evaluation (d)	0.93	.321	0.815–1.069

[a]Abnormal outcome included pregnancy loss, abnormal infant physical exam or abnormal infant neuro-imaging findings.

The nucleic assay used to determine virus load in our studies was the Lanciotti methodology [15]. This is a real-time PCR method used consistently in multiple settings since the ZIKV epidemic in the French Polynesia and in the Americas subsequently. This virus load assay was used in Brazil for identification of cases during the Zika epidemic at the national level [25, 26]. It was also the assay used in our prospective cohort studies of mother-infant pairs with ZIKV infection [10]. Novel PCR methods for Zika have used it as the reference method/gold standard against which novel nucleic acid tests are tested [27]. As future quantitative nucleic acid techniques are developed, it is important to evaluate viral load in serial specimens at low copy levels to ascertain the role of magnitude and duration of viremia in pathogenesis.

We found that severity of maternal clinical findings during primary infection was not associated with congenital ZIKV manifestations. In our ZIKV series, abnormal pregnancy and infant outcomes had a relatively even distribution across patients with mild, moderate and severe clinical manifestations. The lack of association of maternal symptoms with birth outcomes is perhaps not surprising. Other congenital infections, such as maternal rubella, cytomegalovirus (CMV), toxoplasmosis, and syphilis may be clinically unapparent and still cause devastating fetal outcomes.

As rash was an inclusion criterion for subject identification in our study, we had a very small number of patients with mild disease and none with asymptomatic illness, which skewed our severity scores toward moderate to severe manifestations. Even so, our findings substantiate the hypothesis that severity of illness and maternal virus load are not predictive of congenital infection, with fetal abnormal outcomes following asymptomatic disease being very likely. These findings are consistent with a preliminary publication from Colombia that reported cases of ZIKV infection in pregnancy and microcephaly from the National Reporting System data [28]. The authors investigated all cases of microcephaly and found 4 infants with evidence of congenital ZIKV infection that were born to pregnant women with no history of illness in pregnancy. A recent publication by Honein et al. that described outcomes from 442 pregnant women with laboratory evidence of recent ZIKV infection enrolled in the US ZIKV Pregnancy Registry also showed similar data. The authors reported 6% infants or fetuses with birth defects, with an equal proportion of birth defects

following symptomatic or asymptomatic maternal ZIKV infection [29]. Our report provides additional evidence that clinical ZIKV infection symptoms may not correlate with the risk of fetal transmission and do not appear to correlate with abnormal birth outcomes.

We found that gestational age at time of infection is a risk factor for abnormal outcomes in ZIKV-infected pregnant women, albeit the association was borderline significant. This is concordant with our prior analysis of outcomes in this cohort of patients, where 55% of infections in the first trimester resulted in abnormal outcomes, followed by 51% of second trimester infections with abnormal outcomes, and 29% of third trimester infections resulting in abnormal outcomes [10].

Although experimental studies suggest that dengue virus antibodies may enhance ZIKV infection in vitro and hypothetically increase disease severity [13, 14], in our cohort analysis, previous dengue infection was not associated with a higher maternal virus load, neither with disease severity nor abnormal birth outcomes. Conversely, prior dengue antibodies were also not associated with any protective effect against adverse infant outcomes. Although our sample size was limited (only 12% of women had no prior IgG antibodies to dengue), this is an important area of investigation, as it suggests that implementation of dengue immunization programs may likely not adversely affect ZIKV virus pregnancy outcomes.

Our finding of a lack of correlation between maternal clinical symptoms and adverse birth outcomes has significant public health implications with regards to antenatal screening practices for ZIKV infection in asymptomatic pregnant women, particularly in areas where the ZIKV epidemic is ongoing. The Centers for Disease Control and Prevention (CDC) currently recommend testing pregnant women in areas of active ZIKV transmission with immunoglobulin M (IgM) antibodies in the first and second trimester and, if positive at anytime, to confirm by screening urine and serum specimens by PCR [30]. Interpretation of currently available serological tests is complex in areas of dengue endemicity due to the cross reactivity between ZIKV and dengue antibodies. In our cohort, 88% of women, including ZIKV-PCR negative pregnant women, had preexisting dengue antibodies and may have screened positive on IgM testing. Additionally, testing by PCR on serum and urine once in each trimester may miss the period of viremia and/or viruria. A new diagnostic strategy is needed to identify asymptomatic or mild clinical ZIKV infection in pregnancy.

Limitations to our study include the fact that only patients with a rash were enrolled, as that was an inclusion criterion. Therefore, we cannot extrapolate data to patients who were asymptomatic. ZIKV load was not measured in sequential specimens, so the time point of greater viremia or viruria could be missed. For this reason associations with duration of viral shedding could not be evaluated. Absence of prior dengue infection was limited to 12% of women without serologic evidence of dengue, a common problem in endemic areas.

Thus, in conclusion, research efforts for the prevention of perinatal transmission of ZIKV should not be focused on symptomatic patients only. Our finding of no correlation between severity of symptoms in antenatal ZIKV infection and birth outcomes supports the observation that there is risk of congenital ZIKV infection in the absence of symptomatic disease. Development of a reliable, reproducible, sensitive, specific and accessible serologic assay is important to assess the risk of symptomatic and asymptomatic ZIKV infection in pregnancy.

Notes

Acknowledgements. We thank the women and infants who enrolled in this prospective study, and Dr Myung Shim-Sin, PhD, in the Department of Pediatrics at UCLA for assistance with the statistical analysis.

Financial support. This work was supported by the Departamento de Ciência e Tecnologia (DECIT) do Ministério da Saúde do Brasil and grants from Coordenacao de Aperfeicoamento de Pessoal de Nivel Superior (CAPES/ 88887.116627/2016-01); the National Institute of Allergy and Infectious Diseases (NIAID) of the National Institutes of Health (AI28697 and 1R21AI129534-01) and the Thrasher Research Fund (20164370).

Potential conflicts of interest. All authors: No reported conflicts of interest. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References

1. Duffy MR, Chen TH, Hancock WT, et al. Zika virus outbreak on Yap Island, Federated States of Micronesia. N Engl J Med **2009**; 360:2536–43.
2. Lessler J, Ott CT, Carcelen AC, et al. Times to key events in Zika virus infection and implications for blood donation: a systematic review. Bull World Health Organ **2016**; 94:841–9.
3. Musso D, Roche C, Nhan TX, Robin E, Teissier A, Cao-Lormeau VM. Detection of Zika virus in saliva. J Clin Virol **2015**; 68:53–5.
4. Barzon L, Pacenti M, Berto A, et al. Isolation of infectious Zika virus from saliva and prolonged viral RNA shedding in a traveller returning from the Dominican Republic to Italy, January 2016. Euro Surveill **2016**; 21:30159.
5. Campos Rde M, Cirne-Santos C, Meira GL, et al. Prolonged detection of Zika virus RNA in urine samples during the ongoing Zika virus epidemic in Brazil. J Clin Virol **2016**; 77:69–70.
6. Roze B, Najioullah F, Ferge JL, et al. Zika virus detection in urine from patients with Guillain-Barre syndrome on Martinique, January 2016. Euro Surveill **2016**; 21:30154.
7. Bingham AM, Cone M, Mock V, et al. Comparison of test results for Zika virus RNA in urine, serum, and saliva specimens from persons with travel-associated Zika virus disease—Florida, 2016. MMWR Morb Mortal Wkly Rep **2016**; 65:475–8.
8. Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR. Zika virus and birth defects—reviewing the evidence for causality. N Engl J Med **2016**; 374:1981–7.
9. Roberts DJ, Frosch MP. Zika and histopathology in first trimester infections. Lancet **2016**; 388:847–9.
10. Brasil P, Pereira JP Jr, Moreira ME, et al. Zika virus infection in pregnant women in Rio de Janeiro. N Engl J Med **2016**; 375:2321–34.
11. Honein MA, Dawson AL, Petersen EE, et al.; US Zika Pregnancy Registry Collaboration. Birth defects among fetuses and infants of US women with evidence of possible Zika virus infection during pregnancy. JAMA **2017**; 317:59–68.
12. Kleber de Oliveira W, Cortez-Escalante J, De Oliveira WT, et al. Increase in reported prevalence of microcephaly in infants born to women living in areas with confirmed Zika virus transmission during the first trimester of pregnancy—Brazil, 2015. MMWR Morb Mortal Wkly Rep **2016**; 65:242–7.
13. Kawiecki AB, Christofferson RC. Zika virus-induced antibody response enhances dengue virus serotype 2 replication in vitro. J Infect Dis **2016**; 214:1357–60.
14. Dejnirattisai W, Supasa P, Wongwiwat W, et al. Dengue virus sero-cross-reactivity drives antibody-dependent enhancement of infection with Zika virus. Nat Immunol **2016**; 17:1102–8.

15. Lanciotti RS, Kosoy OL, Laven JJ, et al. Genetic and serologic properties of Zika virus associated with an epidemic, Yap State, Micronesia, 2007. Emerg Infect Dis **2008**; 14:1232–9.
16. Soares de Souza A, Moraes Dias C, Braga FD, et al. Fetal infection by Zika virus in the third trimester: report of 2 cases. Clin Infect Dis **2016**; 63:1622–5.
17. Ticconi C, Pietropolli A, Rezza G. Zika virus infection and pregnancy: what we do and do not know. Pathog Glob Health **2016**; 110 262–8.
18. Challis JR, Lockwood CJ, Myatt L, Norman JE, Strauss JF 3rd, Petraglia F. Inflammation and pregnancy. Reprod Sci **2009**; 16:206–15.
19. Burns DN, Nourjah P, Wright DJ, et al. Changes in immune activation markers during pregnancy and postpartum. J Reprod Immunol **1999**; 42:147–65.
20. Denney JM, Nelson EL, Wadhwa PD, et al. Longitudinal modulation of immune system cytokine profile during pregnancy. Cytokine **2011**; 53:170–7.
21. Ohto H, Terazawa S, Sasaki N, et al. Transmission of hepatitis C virus from mothers to infants: the vertical transmission of hepatitis C virus collaborative study group. N Engl J Med **1994**; 330:744–50.
22. Mofenson LM, Lambert JS, Stiehm ER, et al. Risk factors for perinatal transmission of human immunodeficiency virus type 1 in women treated with zidovudine. Pediatric AIDS Clinical Trials Group Study 185 Team. N Engl J Med **1999**; 341:385–93.
23. Burk RD, Hwang LY, Ho GY, Shafritz DA, Beasley RP. Outcome of perinatal hepatitis B virus exposure is dependent on maternal virus load. J Infect Dis **1994**; 170:1418–23.
24. Anderson MJ, Higgins PG, Davis LR, et al. Experimental parvoviral infection in humans. J Infect Dis **1985**; 152:257–65.
25. Brasil P, Calvet GA, Siqueira AM, et al. Zika virus outbreak in Rio de Janeiro, Brazil: clinical characterization, epidemiological and virological aspects. PLoS Negl Trop Dis **2016**;10:e0004636.
26. Calvet G, Aguiar RS, Melo AS, et al. Detection and sequencing of Zika virus from amniotic fluid of fetuses with microcephaly in Brazil: a case study. Lancet Infect Dis **2016**; 16:653–60.
27. Stone M, Lanteri MC, Bakkour S, et al. Relative analytical sensitivity of donor nucleic acid amplification technology screening and diagnostic real-time polymerase chain reaction assays for detection of Zika virus RNA. Transfusion **2017**; 57(3pt2):734–47.
28. Pacheco O, Beltran M, Nelson CA, et al. Zika virus disease in Colombia— preliminary \report. N Engl J Med **2016**: doi: 10.1056/NEJMoa1604037.
29. Honein MA, Dawson AL, Petersen EE, et al.; US Zika Pregnancy Registry Collaboration. Birth defects among fetuses and iinfants of US women with evidence of possible Zika virus infection during pregnancy. JAMA **2017**; 317:59–68.
30. Oduyebo T, Petersen EE, Rasmussen SA, et al. Update: Interim guidelines for health care providers caring for pregnant women and women of reproductive age with possible Zika virus exposure—United States, 2016. MMWR Morb Mortal Wkly Rep **2016**; 65:122–7.