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Zika Virus Infection in Pregnancy, Microcephaly, and Maternal and Fetal Health

What We Think, What We Know, and What We Think We Know

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• **Context.**—The global epidemic of Zika virus (ZIKV) infection has emerged as an important public health problem affecting pregnant women and their infants.

Objectives.—To review the causal association between ZIKV infection during pregnancy and intrauterine fetal infection, microcephaly, brain damage, congenital malformation syndrome, and experimental laboratory models of fetal infection. Many questions remain regarding the risk factors, pathophysiology, epidemiology, and timing of maternal-fetal transmission and disease. These include mechanisms of fetal brain damage and microcephaly; the role of covariables, such as viral burden, duration of viremia, and host genetics, on vertical transmission; and the clinical and pathologic spectrum of congenital Zika syndrome. Additional questions include defining the potential long-term physical and neurobehavioral outcomes for infected infants, whether maternal or fetal host genetics influence the clinical outcome, and whether ZIKV

infection can cause maternal morbidity. Finally, are experimental laboratory and animal models of ZIKV infection helpful in addressing maternal-fetal viral transmission and the development of congenital microcephaly? This communication provides current information and attempts to address some of these important questions.

Data Sources.—Comprehensive review of published scientific literature.

Conclusions.—Recent advances in epidemiology, clinical medicine, pathology, and experimental studies have provided a great amount of new information regarding vertical ZIKV transmission and the mechanisms of congenital microcephaly, brain damage, and congenital Zika syndrome in a relatively short time. However, much work still needs to be performed to more completely understand the maternal and fetal aspects of this new and emerging viral disease.

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The world was stunned when it was first suspected in 2015 that an insect-borne disease associated with a largely unfamiliar virus—the Zika virus (ZIKV)—could cause birth defects in fetuses and newborn infants. On March 2, 2015, Brazil reported to the World Health Organization (WHO) that many cases of an illness, characterized by skin rash, had been occurring in its northeastern states. Patients were initially identified from Pernambuco, Brazil, in December 2014, followed by reports from Rio Grande do Norte, Maranhão, and Bahia, Brazil, in February and March 2015. From February to April 2015, almost 7000 cases of illness characterized by skin rash were reported from these states, but as the ZIKV was not suspected, no tests were conducted for it. On April 29, 2015, the Bahia State Laboratory in Brazil reported to WHO that samples were positive for ZIKV, which was confirmed by polymerase chain reaction (PCR) testing at Brazil's national reference

laboratory on May 7, 2015. On the same day, the WHO and the Pan American Health Organization (PAHO) issued an epidemiologic alert that ZIKV was occurring in Brazil and, for the first time, in the Western hemisphere.¹ By July 2015, Brazil reported the association of ZIKV infection with neurologic disease in adults, including 49 cases of confirmed Guillain-Barré syndrome. In October 2015, Colombia announced that PCR-confirmed cases of ZIKV infection were also occurring in that country.

On October 30, 2015, Brazil first reported an unexplained increase in the number of newborns with microcephaly in the northeastern state of Pernambuco, where there had been a large outbreak of ZIKV infection when the affected children's mothers had been in early pregnancy. Brazil announced on November 11, 2015, that there were 140 cases of suspected congenital microcephaly occurring in Pernambuco State alone, and declared a national public health emergency. The number of Brazilian infants with suspected microcephaly from ZIKV reached more than 700 cases by mid-November 2015, and ZIKV was found in the amniotic fluid of 2 pregnant women. In response, PAHO and WHO issued an epidemiologic alert asking PAHO Member States to report increases of congenital microcephaly and other central nervous system malformations.² On November 28, 2015, Brazil reported that ZIKV genomic material had been isolated from both tissue and blood

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specimens from an infant with congenital abnormalities, including microcephaly, who died within minutes after delivery. Following this report, both PAHO and WHO issued an alert to the association of ZIKV infection with neurologic syndromes and congenital malformations in the Americas.³

The number of suspected congenital microcephaly cases associated with ZIKV in Brazil had increased to 2975 by January 2016, and new cases of adult ZIKV infection were being reported from countries throughout the Caribbean and Central and South America. On January 5, 2016, the first cases of intrauterine transmission of ZIKV were described from 2 pregnant women in Brazil whose fetuses were diagnosed by ultrasonography as having microcephaly and severe brain abnormalities.⁴ Three infants with severe congenital ocular abnormalities occurring with microcephaly were reported on January 7, 2016, from Brazil, widening the spectrum of fetal malformations associated with the ZIKV.⁵ On January 12, 2016, the US Centers for Disease Control and Prevention (CDC) in Atlanta, Georgia, working in collaboration with health officials in Brazil, released information regarding the occurrence of 2 neonatal deaths with congenital microcephaly and 2 miscarriages from Brazil, all of whom tested positive for ZIKV using reverse transcriptase-polymerase chain reaction (RT-PCR) and immunohistochemical techniques.⁶ The WHO declared that the occurrence of maternal ZIKV infections, together with clusters of microcephaly and other neurologic disorders, constituted a Public Health Emergency of International Concern on February 1, 2016.⁷ During the following weeks, additional cases of maternal ZIKV infection and infants with congenital microcephaly were identified, including detailed reports of autopsies of fetuses and neonates with microcephaly and intrauterine infections,^{8,9} which strengthened the connection between ZIKV infection and fetal malformations. Any remaining doubts about the relationship between the virus and the consequences of intrauterine infection were eliminated on April 13, 2016, when investigators from the CDC reported that sufficient evidence had accumulated to infer a causal association between prenatal ZIKV infection, microcephaly, and other fetal malformations.¹⁰

Many questions remain unanswered: Exactly how is ZIKV transmitted to the fetus? Does the placenta protect the fetus or facilitate transmission? Do viral intrinsic factors and viral loads have a role in vertical transmission? How soon after maternal infection does vertical transmission occur? Under what circumstances, and what are the effects of, comorbidities on maternal and fetal disease? Moreover, when, during pregnancy, is vertical infection most likely to produce fetal malformations? What constitutes the clinicopathologic spectrum of congenital Zika congenital syndrome? Do experimental laboratory and animal models of ZIKV infection aid the understanding of congenital infection? Additional information needed includes defining the mechanisms for fetal brain damage and other congenital abnormalities, determining whether ZIKV can directly damage organs outside of the fetal brain, and understanding how often fetal infections occur without the development of malformations. Clinical implications also remain unknown, including whether early fetal infections can be diagnosed before the development of malformations, the potential long-term physical and neurobehavioral outcomes for infected infants, whether maternal or fetal host genetics influence the clinical outcome, and whether ZIKV infection

can cause maternal morbidity. This review provides current information and attempts to address some of these important questions.

MICROCEPHALY AND FETAL BRAIN DAMAGE

Before the 2015 epidemic, ZIKV was considered to be of limited public health importance, even though an outbreak had recently been occurring in the islands of French Polynesia. However, in 2015, Brazilian physicians observed dramatic increases in the occurrence of microcephaly, as well as severe cerebral atrophy, ventriculomegaly, and intracranial calcifications, during routine obstetric ultrasound and after delivery. As more clinical, pathologic, epidemiologic, and experimental data accumulated and were analyzed, the CDC formally declared, in April 2016, that a causal relationship existed between prenatal ZIKV infection and microcephaly and brain abnormalities.¹⁰ Retrospective analysis of the ZIKV outbreak that had occurred in French Polynesia between October 2013 and April 2014 revealed that there were 8 cases of congenital microcephaly, which represented a risk of approximately 1%, when maternal infection occurred in the first trimester.^{11,12}

Microcephaly is defined as an occipitofrontal circumference below the third percentile or greater than 2 SD from the reference range distribution; *severe microcephaly* is defined clinically as an occipitofrontal circumference of greater than 3 SD.¹³ Microcephaly, together with most other congenital abnormalities, has complex causal mechanisms and often has multifactorial etiologies. The initial clue to determining ZIKV as the causal agent for the reported cases of microcephaly in Brazil was the strong spatiotemporal relationship, which enabled more resources to be devoted to research and to uncovering further evidence.

Pathology studies have made a significant contribution to both the understanding of the anatomic abnormalities present in fetuses and infants with microcephaly caused by the virus and to the eventual recognition of the causative relationship between intrauterine ZIKV infection and brain damage.^{8,14} On February 10, 2016, Mlakar and colleagues¹⁵ reported the results of a comprehensive autopsy analysis from a fetus with presumed microcephaly due to symptomatic maternal ZIKV infection acquired in Brazil. At the time of postmortem examination, after elective termination of the pregnancy, the fetus, at 32-weeks gestation, was found to have microcephaly, hydrocephalus, an abnormally small brain (microcephaly) with almost complete agyria, and multiple microscopic abnormalities, including multifocal dystrophic calcifications in the cortex and subcortical white matter, cortical displacement, and focal inflammation. Electron microscopy revealed flavivirus-like particles. Significantly, ZIKV was identified in the fetal brain tissue with an RT-PCR assay. At the same time, Martines et al^{16,17} provided their preliminary report of the results of pathology examinations of the brains of 2 newborns (36 and 38 weeks gestation) with microcephaly, who had died less than 24 hours after birth, and 2 miscarriages at 11 and 13 weeks gestation, all from Brazil. All 4 pregnant mothers had clinical signs of ZIKV infection during their first trimesters. Autopsy examination of the brains of the 2 neonates revealed multiple pathologic findings, including parenchymal calcifications, microglial nodules, gliosis, and cellular degeneration and necrosis. Lissencephaly was present in the brains of both infants, and 1 also had holoprosencephaly and

cerebellar hypoplasia. All 4 cases were positive for the virus, with RT-PCR detection of viral RNA from either brain or placental tissues. In an analysis of 9 reported autopsies of fetuses and infants with laboratory-confirmed congenital ZIKV infection and microcephaly, Schwartz⁹ found an overlapping spectrum of viral-induced, gross and microscopic abnormalities in the brains. The microscopic neuropathologic changes among these autopsies consisted of a combination of necrosis that preferentially targeted neurons, degenerative changes of the glial and neuronal cells, white matter loss and axonal rarefaction, microcalcifications, microglial aggregates, gliosis, macrophage and mononuclear inflammatory cell infiltrates, neuronophagy, and perivascular lymphocytic cuffing. In addition, concordance was seen among these autopsies, not only in the pathologic spectrum of brain injury but also in the lack of findings of direct, viral-induced cytopathic effects in organs and tissues outside of the brain.

CONGENITAL ZIKA SYNDROME

Although microcephaly has received most of the attention as a pregnancy-related consequence of ZIKV infection, an expanding spectrum of fetal malformations associated with intrauterine ZIKV infection continues to be described. Currently referred to as *congenital Zika syndrome (CZS)*, this new congenital malformation syndrome includes not only microcephaly and fetal brain damage, but also a range of developmental abnormalities, including musculoskeletal, ocular, craniofacial, genitourinary, pulmonary, and other manifestations (Table). Intrauterine growth restriction also occurs in infants with CZS. Because of this constellation of findings, CZS has a great deal in common with those infectious agents that are responsible for the TORCH syndrome (toxoplasmosis, rubella, cytomegalovirus, and herpes simplex), and thus, some investigators believe that the ZIKV can be considered a TORCH agent (unpublished data, Schwartz DA, August 2016).

Clinical, pathologic, and experimental studies have established that ZIKV is strongly neurotropic, targeting not only neural progenitor cells but also neurons and other brain cells. This direct, viral-induced brain damage can account for many of the malformations observed in cases of CZS, including those of fetal akinesia deformation sequence (FADS), such as decreased fetal activity, craniofacial abnormalities, joint contractures, limb malformations and arthrogryposis, pulmonary hypoplasia, and cryptorchidism.^{9,17,18} Similar to other congenital malformation syndromes, including those produced by some TORCH agents, variability exists in both the spectrum and the severity of the embryopathy in infants with CZS. In fact, there is recent evidence that the use of standard criteria for microcephaly may be insufficient to detect all cases of CZS. In a study from Brazil, França et al¹⁹ reported that 1 in 5 newborns with probable or definite CZS had head circumferences that are within reference range.

Ocular Abnormalities

Following the initial surge of recognized microcephaly cases, reports appeared suggesting that ocular abnormalities occurred in congenital ZIKV infection. Among the early reports, Ventura et al⁵ described 3 Brazilian infants who were born to symptomatic mothers and who each had congenital microcephaly and abnormalities in the macular region of one eye, characterized by pigment mottling and

Categories of Malformations Associated With Congenital Zika Syndrome	
Category	Examples
Neurologic	<ul style="list-style-type: none"> • Microcephaly • Hydrocephalus • Micrencephaly • Lissencephaly • Polymicrogyria • Pachygyria • Agyria • Holoprosencephaly • Ventriculomegaly • Corpus callosum abnormalities • Intracerebral calcifications • Destructive brain lesions
Ocular	<ul style="list-style-type: none"> • Chorioretinal atrophy • Optic nerve abnormalities • Maculopathies • Vascular abnormalities
Musculoskeletal	<ul style="list-style-type: none"> • Arthrogryposis • Craniofacial abnormalities (craniosynostosis) • Clubfoot • Acetabular dysplasia
Genitourinary	<ul style="list-style-type: none"> • Cryptorchidism • Hypospadias
Other	<ul style="list-style-type: none"> • Intrauterine growth restriction • Anasarca • Pulmonary hypoplasia • Single umbilical artery

loss of foveal reflex. Only one of these children presented with well-defined macular atrophy. In a more extensive study by Ventura et al²⁰ with 10 infants with microcephaly and presumed ZIKV infection, 3 of the children had eye exams within reference range, whereas the other 7 had a range of ocular abnormalities that included optic nerve hypoplasia, optic nerve double-ring sign, optic nerve head pallor, alterations in cup to disk ratio, foveal reflex loss, pigment mottling that ranged from mild to gross, and chorioretinal macular atrophy. In another Brazilian case series, which included 29 infants with microcephaly and presumed CZS, more than one-third of the children had ocular abnormalities.²¹ In a report of 40 infants with microcephaly and presumed CZS from Pernambuco, Brazil, Ventura et al²² described ophthalmoscopic alterations in 55% (n = 22) of the infants.

The spectrum of ocular abnormalities reported in CZS includes maculopathies, such as a particular pattern of macular chorioretinal atrophy with a hyperpigmented ring that resembles torpedo maculopathy, abnormalities of the optic nerve (hypoplasia and severe cupping of the optic disk), microcornea, microphthalmia, falciform folds, pigmentary and hemorrhagic retinopathy, circumscribed chorioretinal atrophy, abnormal vascular development (tortuosity, early termination, absence), coloboma, lens subluxation, cataracts, and retinal dysplasia.^{21–25}

Musculoskeletal, Genitourinary, and Pulmonary Abnormalities

Musculoskeletal abnormalities are frequently described in published reports of CZS, but their actual prevalence among infants of women infected during pregnancy remains unknown. Schwartz⁹ analyzed 9 reported autopsies of fetuses and infants with intrauterine ZIKV infection and

congenital microcephaly; of which, 6 had variable findings of craniofacial malformations, craniosynostosis, and limb abnormalities, which included contractures of multiple joints or arthrogryposis. In an additional autopsy performed in Spain, a 19-week fetus with ZIKV infection from a symptomatic mother underwent an autopsy, which revealed arthrogryposis multiplex congenita, consisting of flexion contracture and deformity of the joints of all 4 limbs, severe flexion of the hips, and crossed femurs.²⁶ The skeletal muscles were underdeveloped and had undergone fatty replacement, and there was fibrosis involving the inter-articular spaces. The fetus also had hydrocephalus, dilatation of both lateral ventricles, and cerebral calcifications but no microcephaly.

The musculoskeletal findings and malformations present in CZS are probably not the result of direct cytopathic effect of the virus on the affected tissues, but instead are likely secondary to ZIKV-induced brain injury.^{9,17} Reduction in fetal mobility resulting from intrauterine infection occurring in early development is a well-known cause of arthrogryposis, fetal akinesia deformation sequence, and related malformations.

Additional abnormalities have been reported in association with CZS, including coronal hypospadias, bilateral cryptorchidism, respiratory distress, hypoglycemia, both increased muscle tone and mild hyperreflexia as well as decreased muscle tone, bilateral acetabular dysplasia, amyoplasia of the lower limbs, and pulmonary hypoplasia.^{17,23,27} Data from Colombia suggests that genitourinary, digestive and cardiac systems may also be affected by CZS.¹⁸ In the future, as additional cases of fetal and neonatal infection are identified, and clinical follow-up of the children progresses, new abnormalities will most likely continue to be recognized as components of the CZS.

EXPERIMENTAL INFECTION, NEUROTROPISM, AND NEURONAL DAMAGE

Experimental laboratory testing has been important in understanding and explaining the cellular basis for development of microcephaly in fetuses infected with ZIKV. The use of in vitro cellular systems and in vivo animal models has been especially significant because autopsies of fetuses and infants with congenital ZIKV infection are concordant in demonstrating microcephaly and a spectrum of gross and microscopic changes to the brain, indicative of a high level of selective neurotropism by the virus.^{8,9,14}

Investigators have successfully used in vitro experimental models of neural cell precursors, termed *neurospheres* and *brain organoids*, to study the predilection of ZIKV for neurons. These systems have been made possible by recent advances in the development of induced pluripotent stem cell (iPSC) and embryonic stem cell (ESC) technologies.²⁸ The stem cells have been successfully differentiated toward regional subtypes of neurons of the midbrain, forebrain, and hindbrain, as well as the formation of 3-dimensional organoid systems, allowing researchers to study a variety of neural diseases, including microcephaly.²⁹ Neurospheres and brain organoids are useful in vitro models for investigating developmental neuropathologic changes and have been especially informative in helping researchers understand how the ZIKV affects the fetal brain. Garcez and colleagues³⁰ used human neural stem cells, neurospheres, and brain organoids to demonstrate that ZIKV, but not the related flavivirus dengue virus, induced cell death in neural

stem cells, disrupted the formation of neurospheres, and reduced the growth of organoids. The ZIKV-infected neurospheres had apoptotic nuclei and contained viral particles that were bound to the membranes and were observed in both mitochondria and vesicles of the cells within infected neurospheres. Using a similar system of embryonic stem cell-derived cerebral organoids, Dang and colleagues³¹ demonstrated that ZIKV could produce infection in these cells, which resulted in the decreased size of the organoids, and that the size of the organoids correlated with the viral copy number. They also found that ZIKV infection affected the innate immune receptor toll like receptor 3 (*TLR3*), which has previously been implicated in neuroinflammatory and neurodegenerative conditions. *TLR3* was upregulated following ZIKV infection of human organoids and mouse neurospheres, and inhibition of *TLR3* decreased the phenotypic effects of viral infection. A pathway analysis of changes in gene expression during *TLR3* activation showed 41 genes were related to neuronal development, indicative of a potential mechanistic connection to disrupted neurogenesis. Qian and colleagues³² developed a forebrain-specific organoid system derived from human-induced pluripotent stem cells to model ZIKV exposure. They produced productive viral infections in these progenitor cells, which caused reduced cellular proliferation and increased cell death, resulting in diminished neuronal cell volume, resembling microcephaly. Tang et al³³ found that ZIKV can infect human neural progenitor cells and, significantly, release infectious Zika particles. In addition, they demonstrated that ZIKV caused transcriptional dysregulation, which primarily involved cell cycle-related pathways and increased cell death, causing attenuation of human neural progenitor cell growth.

Animal models of Zika infection, including mice and rhesus macaques, have also proven useful in the understanding of the pathophysiology of viremia, brain damage, and microcephaly.³⁴⁻³⁷ Li et al³⁸ observed that experimental infection of infected embryonic mouse brains with ZIKV resulted in smaller brain size with enlarged lateral ventricles and thinner cortical plates when compared with uninfected controls. In this model, the neural progenitor cells and intermediate or basal progenitor cells were the primary cells affected. Infection of the neural progenitor cells caused high levels of viral replication and attenuation of neural progenitor cell expansion via viral-induced apoptosis and cell cycle dysregulation.³⁸ In the mouse model developed by Lazear et al,³⁹ triple knockout mice (*Irf3^{-/-}Irf5^{-/-}Irf7^{-/-}*), which produce little interferon α/β , and mice lacking the interferon receptor (*Ifnar1^{-/-}*), developed neurologic disease and succumbed to ZIKV infection after experimental inoculation. The (*Ifnar1^{-/-}*) mice developed high viral burdens in the brain, spinal cord, and testes. Cugola and colleagues⁴⁰ administered ZIKV to pregnant mice in midgestation and produced some animals with stunted heads, intrauterine growth restriction, and ocular abnormalities. Wu et al⁴¹ performed intraperitoneal inoculation of pregnant mice with ZIKV, producing viremia, placental infection, and subsequent viral infection of the radial glial cells of the dorsal ventricular zone of the fetal brain. This reduced the number of radial glial cells, the primary neural cells responsible for the development of the cortex, and consequently, decreased the surface area of the cortex and the lateral ventricles. Animal models of ZIKV infection should prove valuable to not only define mechanisms of

fetal brain injury but also improve future development and testing of vaccines.

TIMING OF INTRAUTERINE TRANSMISSION

It was initially uncertain whether ZIKV could be transmitted from an infected mother to her fetus with equal efficacy throughout gestation or whether there was a period during which vertical transmission had a higher risk of occurrence. It is critically important to know when during gestation maternal ZIKV infection produces the highest risk of transmission to the fetus, similar to that identification for other TORCH agents, such as cytomegalovirus and rubella. In the case of rubella, the risk of adverse fetal outcomes is greatest when infection occurs during the first trimester; for cytomegalovirus, the risk is highest during the first trimester but is also present after exposure during the second or third trimesters.^{42,43} The timing of vertical ZIKV transmission is an important public health issue because it helps guide policy and public health recommendations for delaying pregnancy following maternal infection, implementation of prenatal diagnostic considerations, and providing guidance for any future treatment options. Recent clinical studies have provided important preliminary evidence that maternal infection by ZIKV early in the pregnancy is associated with the greatest risk of mother-to-fetus transmission and the development of microcephaly. Preliminary evidence from Brazil has indicated that the development of microcephaly correlates best with maternal viral infection occurring at approximately 17 weeks gestation.⁴⁴ A peak risk for the development of microcephaly when Zika infection occurs in the first trimester of pregnancy was found in a study in Bahia, Brazil, by Johansson et al,⁴⁵ who found that there was a negligible risk of development of the CZS when maternal infection developed in the second and third trimesters. They also estimated that the probability of having a fetus with microcephaly varied from approximately 1% to 13% when a woman was infected with ZIKV during the first trimester. In Colombia, Pacheco and colleagues²⁷ enrolled a group of 1850 pregnant women with RT-PCR-confirmed ZIKV infection in a study for whom complete data on the trimester of infection were available—532 women (28.7%) acquiring the infection in the first trimester, 702 (37.9%) in the second trimester, and 616 (33.3%) in the third trimester. During the period from January 1, 2016, to April 28, 2016, there were no infants with microcephaly or other brain abnormalities born to those mothers who had acquired ZIKV infection in the third trimester, providing additional evidence for the significance of infection early in gestation causing brain abnormalities.

It remains unknown whether maternal viremia is a significant covariate in the occurrence and timing of vertical transmission of ZIKV infection. Initially, it was believed that after maternal infection, ZIKV circulated in the mother's bloodstream for a brief period, averaging 9.9 days.⁴⁶ However, a case report of prolonged material viremia of approximately 10 weeks after initial symptoms was described⁴⁷ with a fetal outcome of microcephaly, and ZIKV was present in the fetal brain at the time of autopsy. In a recent report of a pregnant woman who developed a ZIKV infection during the first trimester and had a miscarriage at 11 weeks, ZIKV was identified in her serum 21 days after the onset of symptoms. Zika virus was present in the fetal tissue, amniotic fluid, and placenta.⁴⁸ Meaney-Delman and colleagues⁴⁹ have described ZIKV present in the serum of 4

symptomatic pregnant women up to 46 days after the onset of maternal symptoms, and 53 days after exposure in an asymptomatic, pregnant woman. An interesting observation was that, of the 4 infants delivered by the time of their report, only 1 was born with fetal infection—that mother had ZIKV viremia documented for 17 days. Three of the infants delivered were apparently healthy (following maternal viremia for 23, 46, and 53 days after exposure or onset of symptoms). In the one woman in this cohort who had not yet delivered, persistent viremia was present 44 days after symptom onset, and the fetus had ultrasonographic evidence of a head circumference in the sixth percentile at 38 weeks gestation.⁴⁹

MATERNAL MORBIDITY AND ZIKA VIRUS

The effects of ZIKV infection on the fetus are the subject of intense investigation; however, the risks of morbidity and mortality to the mother during pregnancy are not well known. The symptoms of ZIKV in pregnant women appear similar to those in nonpregnant adults. A recent study⁵⁰ compared 72 pregnant women with symptomatic ZIKV infections and 16 ZIKV-negative, pregnant women in Brazil. The most common findings in the ZIKV-infected women included maculopapular rash in all 72 women (100%), pruritus affecting 69 women (95.8%), arthralgia in 46 (63.9%), and conjunctival injection in 42 (58.3%). Fever was present in 20 of the 72 women (27.8%), which was typically low grade and short term. Interestingly, there was a significantly higher frequency of lymphadenopathy (29 of 72; 40.3%) and conjunctival injection (42 of 72; 58.3%) in ZIKV-infected pregnant women compared with uninfected pregnant women (1 of 15 [6.7%] and 2 of 15 [13.3%], respectively), but the significance of this disparity is unknown. Two of the ZIKV-infected women (2 of 72; 2.8%) had miscarriages during the first trimester in this study.⁵⁰

Thus far, there has been one report, to our knowledge, of ZIKV-induced maternal morbidity from neurologic disease. In that case, maternal Guillain-Barré syndrome occurred at 28-weeks gestation with the development of respiratory distress that required admission to an intensive care unit. The woman recovered completely within 3 weeks and delivered a healthy infant after 39 weeks of gestation.⁵¹

Although the medical complications of ZIKV infection occurring in nonpregnant adults were initially believed to be limited to Guillain-Barré syndrome, recent evidence has shown that a variety of disease entities may result from infection in adults. Other neurologic manifestations that have been reported in association with ZIKV infection include meningoencephalitis and acute myelitis.^{52–54} These neurologic manifestations have been expanded to include encephalitis, paresthesia, vertigo, facial paralysis, and auditory manifestations that have been described as dull, metallic hearing.^{53,55,56} Nonneurologic complications in adults are extremely rare but include immune-mediated thrombocytopenia; postinfectious chronic arthritis; and possible association with chronic fatigue syndrome.^{52,56–58} Patients with malaria coinfection or sickle cell anemia have also been reported to develop jaundice with ZIKV infection.⁵⁶

Zika virus infection has a low fatality rate in adults, and those few reported cases of adult death associated with the virus have occurred in patients with coexisting conditions, such as lupus and sickle cell anemia.^{56,59} However, because

many of the complications occurring in adults with ZIKV infections have comorbid risk factors that are present in pregnant women, there will most likely be future cases of ZIKV-associated maternal morbidity.

References

1. Pan American Health Organization (PAHO); World Health Organization. Zika virus infection: epidemiological alert [press release]. Washington, DC: PAHO; May 7, 2015. http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=30075=&en. Accessed August 4, 2016.
2. Pan American Health Organization (PAHO); World Health Organization. Increase of microcephaly in the northeast of Brazil—increase of microcephaly in the northeast of Brazil: epidemiological alert [press release]. Washington, DC: PAHO; November 17, 2015. http://www.paho.org/hq/index.php?option=com_docman&task=doc_view&Itemid=270&gid=32285=&en. Accessed June 10, 2016.
3. Pan American Health Organization (PAHO); World Health Organization. Neurological syndrome, congenital malformations, and Zika virus infection—implication for public health in the Americas: epidemiological alert [press release]. Washington, DC: PAHO; December 1, 2015. http://www.paho.org/hq/index.php?option=com_docman&task=doc_download&Itemid=&gid=32405=&en. Accessed June 10, 2016.
4. Oliveira Melo AS, Malinger G, Ximenes R, Szejnfeld PO, Alves Sampaio S, Bispo de Filippis AM. Zika virus intrauterine infection causes fetal brain abnormality and microcephaly: tip of the iceberg? *Ultrasound Obstet Gynecol*. 2016;47(1):6–7.
5. Ventura CV, Maia M, Bravo-Filho V, Góis AL, Belfort R Jr. Zika virus in Brazil and macular atrophy in a child with microcephaly [correspondence]. *Lancet*. 2016;387(10015):228. doi:10.1016/S0140-6736(16)00066-4.
6. Branswell H. Zika virus likely tied to Brazil's surge in babies born with small heads, CDC says. *STAT*. January 13, 2016. <http://www.statnews.com/2016/01/13/zika-brazil-cdc-testing/>. Accessed June 20, 2016.
7. World Health Organization. WHO director-general summarizes the outcome of the Emergency Committee regarding clusters of microcephaly and Guillain-Barré syndrome. <http://www.who.int/mediacentre/news/statements/2016/emergency-committee-zika-microcephaly/en/>. Published February 1, 2016. Accessed June 15, 2016.
8. Schwartz DA. *Fetal Brain Damage and Zika Virus Infection: A Strengthening Etiologic Link Following Post-Mortem Examinations*. Washington, DC: Springer Nature. *Expert Commentaries on the Zika Virus*. <http://www.springernature.com/gp/group/zika-virus/how-pathology-helps-to-understand-the-role-of-zika-virus-during-10016228>. Published April 15, 2016. Accessed July 5, 2016.
9. Schwartz DA. Autopsy and postmortem studies are concordant: pathology of Zika virus infection in neonates and stillborn fetuses with microcephaly following transplacental transmission [published online ahead of print August 24, 2016]. *Arch Pathol Lab Med*. 2016. doi:10.5858/arpa.2016-0343-OA.
10. Rasmussen SA, Jamieson DJ, Honein MA, Petersen LR. Zika virus and birth defects—reviewing the evidence for causality. *N Engl J Med*. 2016;374(20):1981–1987. doi:10.1056/NEJMs1604338.
11. Cao-Lormeau VM, Blake A, Mons S, et al. Guillain-Barré Syndrome outbreak associated with Zika virus infection in French Polynesia: a case-control study. *Lancet*. 2016;387(10027):1531–1539. doi:10.1016/S0140-6736(16)00562-6.
12. Cauchemez S, Besnard M, Bompard P, et al. Association between Zika virus and microcephaly in French Polynesia, 2013–15: a retrospective study. *Lancet*. 2016;387(10033):2125–2132. doi:10.1016/S0140-6736(16)00651-6.
13. Panchaud A, Stojanov M, Ammerdorffer A, Vouga M, Baud D. Emerging role of Zika virus in adverse fetal and neonatal outcomes. *Clin Microbiol Rev*. 2016;29(3):659–694. doi:10.1128/CMR.00014-16.
14. Schwartz DA. *How Pathology Helps to Understand the Role of Zika Virus During Pregnancy and Fetal Infection*. Washington, DC: Springer Nature. *Expert Commentaries on the Zika Virus*. <http://www.springernature.com/gp/group/zika-virus/how-pathology-helps-to-understand-the-role-of-zika-virus-during-7823014>. Published March 14, 2016. Accessed June 30, 2016.
15. Mlakar J, Korva M, Tul N, et al. Zika virus associated with microcephaly. *N Engl J Med*. 2016;374(10):951–958. doi:10.1056/NEJMoa1600651.
16. Martines RB, Bhatnagar J, Keating MK, et al. Notes from the field: evidence of Zika virus infection in brain and placental tissues from two congenitally infected newborns and two fetal losses—Brazil, 2015. *MMWR Morb Mortal Wkly Rep*. 2016;65(6):159–160. doi:10.1585/mmwr.mm6506e1.
17. Martines RB, Bhatnagar J, Ramos AMO, et al. Pathology of congenital Zika syndrome in Brazil: a case series [published online ahead of print June 29, 2016]. *Lancet*. doi:10.1016/S0140-6736(16)30883-2.
18. Costello A, Dua T, Duran P, et al. Defining the syndrome associated with congenital Zika virus infection. *Bull World Health Organ*. 2016;94(6):406–406A. doi:10.2471/BLT.16.176990.
19. França GV, Schuler-Faccini L, Oliveira WK, et al. Congenital Zika virus syndrome in Brazil: a case series of the first 1501 livebirths with complete investigation [published online ahead of print June 29, 2016]. *Lancet*. doi:10.1016/S0140-6736(16)30902-3.
20. Ventura CV, Maia M, Ventura BV, et al. Ophthalmological findings in infants with microcephaly and presumed intra-uterine Zika virus infection. *Arg Bras Oftalmol*. 2016;79(1):1–3. doi:10.5935/0004-2749.20160002.
21. de Paula Freitas B, de Oliveira Dias JR, Prazeres J, et al. Ocular findings in infants with microcephaly associated with presumed Zika virus congenital infection in Salvador, Brazil. *JAMA Ophthalmol*. 2016;134(5):529–535.
22. Ventura C, Maia M, Travassos SB, et al. Risk factors associated with the ophthalmoscopic findings identified in infants with presumed Zika virus congenital infection [published online ahead of print August 1, 2016]. *JAMA Ophthalmol*. 2016;134(8):912–918. doi:10.1001/jamaophthalmol.2016.1784.
23. Culjat M, Darling SE, Nerurkar VR, et al. Clinical and imaging findings in an infant with Zika embryopathy [published online ahead of print May 18, 2016]. *Clin Infect Dis*. doi:10.1093/cid/ciw324.
24. Miranda HA II, Costa MC, Frazão MA, Simão N, Franchischini S, Moshfeghi DM. Expanded spectrum of congenital ocular findings in microcephaly with presumed Zika infection. *Ophthalmology*. 2016;123(8):1788–1794. doi:10.1016/j.ophtha.2016.05.001.
25. Panchaud A, Stojanov M, Ammerdorffer A, Vouga M. Emerging role of Zika virus in adverse fetal and neonatal outcomes. *Clin Microbiol Rev*. 2016;29(3):659–694. doi:10.1128/CMR.00014-16.
26. Perez S, Tato R, Cabrera JJ, et al. Confirmed case of Zika virus congenital infection, Spain, March 2016. *Euro Surveill*. 2016;21(24):1–4. doi:10.2807/1560-7917.ES.2016.21.24.30261.
27. Pacheco O, Beltrán M, Nelson CA, et al. Zika virus disease in Colombia—preliminary report [published online ahead of print June 15, 2016]. *N Engl J Med*. doi:10.1056/NEJMoa1604037. <http://www.nejm.org/doi/full/10.1056/NEJMoa1604037#t=article>. Accessed July 16, 2016.
28. Yamanaka S. Induced pluripotent stem cells: past, present, and future. *Cell Stem Cell*. 2012;10(6):678–684. doi:10.1016/j.stem.2012.05.005.
29. Lancaster MA, Renner M, Martin CA, et al. Cerebral organoids model human brain development and microcephaly. *Nature*. 2013;501(7467):373–379. doi:10.1038/nature12517.
30. Garcez PP, Lioia EC, Madeiro da Costa R, et al. Zika virus impairs growth in human neurospheres and brain organoids. *Science*. 2016;352(6287):816–818. doi:10.1126/science.aaf6116.
31. Dang J, Tiwari SK, Lichinchi G, et al. Zika virus depletes neural progenitors in human cerebral organoids through activation of the innate immune receptor TLR3 [published online ahead of print May 6, 2016]. *Cell Stem Cell*. 2016;19(2):258–265. doi:10.1016/j.stem.2016.04.014.
32. Qian X, Nguyen HN, Song MM, et al. Brain-region-specific organoids using mini-bioreactors for modeling ZIKV exposure. *Cell*. 2016;165(5):1238–1254. doi:10.1016/j.cell.2016.04.032.
33. Tang H, Hammack C, Ogden SC, et al. Zika virus infects human cortical neural progenitors and attenuates their growth. *Cell Stem Cell*. 2016;18(5):587–590. doi:10.1016/j.stem.2016.02.016.
34. Brault AC, Bowen RA. The development of small animal models for Zika virus vaccine efficacy testing and pathological assessment. *Am J Trop Med Hyg*. 2016;94(6):1187–1188. doi:10.4269/ajtmh.16-0277.
35. Rossi SL, Tesh RB, Azar SR, et al. Characterization of a novel murine model to study Zika virus. *Am J Trop Med Hyg*. 2016;94(6):1362–1369. doi:10.4269/ajtmh.16-0111.
36. Aliota MT, Caine EA, Walker EC, Larkin KE, Camacho E, Osorio JE. Characterization of lethal Zika virus infection in AG129 mice [published correction appears in *PLoS Negl Trop Dis*. 2016;10(5):e0004750]. *PLoS Negl Trop Dis*. 2016;10(4):e0004682. doi:10.1371/journal.pntd.0004682.
37. Dudley DM, Aliota MT, Mohr EL, et al. A rhesus macaque model of Asian-lineage Zika virus infection. *Nat Commun*. 2016;7:12204. doi:10.1038/ncomms12204.
38. Li C, Xu D, Ye Q, et al. Zika virus disrupts neural progenitor development and leads to microcephaly in mice. *Cell Stem Cell*. 2016;19(1):120–126. doi:10.1016/j.stem.2016.04.017.
39. Lazear HM, Govero J, Smith AM, et al. A mouse model of Zika virus pathogenesis. *Cell Host Microbe*. 2016;19(5):720–730. doi:10.1016/j.chom.2016.03.010.
40. Cugola FR, Fernandes IR, Russo FB, et al. The Brazilian Zika virus strain causes birth defects in experimental models. *Nature*. 2016;534(7606):267–271. doi:10.1038/nature18296.
41. Wu KY, Zuo GL, Li XF, et al. Vertical transmission of Zika virus targeting the radial glial cells affects cortex development of offspring mice. *Cell Res*. 2016;26(6):645–654. doi:10.1038/cr.2016.58.
42. Miller E, Cradock-Watson JE, Pollock TM. Consequences of confirmed maternal rubella at successive stages of pregnancy. *Lancet*. 1982;2(8302):781–784.
43. Pass RF, Fowler KB, Boppa SB, Britt WJ, Stagno S. Congenital cytomegalovirus infection following first trimester maternal infection: symptoms at birth and outcome. *J Clin Virol*. 2006;35(2):216–220.
44. Faria NR, Azevedo RSS, Kraemer MU, et al. Zika virus in the Americas: early epidemiological and genetic findings. *Science*. 2016;352(6283):345–349. doi:10.1126/science.aaf5036.
45. Johansson MA, Mier-y-Teran-Romero L, Reefhuis J, Gilboa SM, Hills SL. Zika and the risk of microcephaly. *N Engl J Med*. 2016;375(1):1–4. doi:10.1056/NEJMp1605367.
46. Lessler JT, Ott CT, Carcelen AC, et al. Times to key events in the course of Zika infection and their implications: a systematic review and pooled analysis [published online ahead of print April 1, 2016]. *Bull World Health Organ*. doi:10.2471/BLT.16.174540. http://www.who.int/bulletin/online_first/16-174540.pdf. Accessed August 4, 2016.

47. Driggers RW, Ho CY, Korhonen EM, et al. Zika virus infection with prolonged maternal viremia and fetal brain abnormalities. *N Engl J Med*. 2016; 374(22):2142–2151. doi:10.1056/NEJMoa1601824.
48. van der Eijk AA, van Genderen PJ, Verdijk RM, et al. Miscarriage associated with Zika virus infection [letter; published online ahead of print July 27, 2016]. *N Engl J Med*. doi:10.1056/NEJMc1605898.
49. Meaney-Delman D, Oduyebo T, Polen KN, et al; U.S. Zika Pregnancy Registry Prolonged Viremia Working Group. Prolonged detection of Zika virus RNA in pregnant women [published online ahead of print July 29, 2016]. *Ob Gyn*. doi:10.1097/AOG.0000000000001625.
50. Brasil P, Pereira JP Jr, Raja Gabaglia C, et al. Zika virus infection in pregnant women in Rio de Janeiro—preliminary report [published online ahead of print March 4, 2016]. *N Engl J Med*. doi:10.1056/NEJMoa1602412.
51. Reyna-Villasmil E, López-Sánchez G, Santos-Bolivar J. Guillain-Barré syndrome due to Zika virus during pregnancy [in Spanish]. *Med Clin (Barc)*. 2016;146(7):331–332.
52. Hajra A, Bandyopadhyay D, Hajra SK. Zika virus: a global threat to humanity: a comprehensive review and current developments. *N Am J Med Sci*. 2016;8(3):123–128. doi:10.4103/1947-2714.179112.
53. Petersen LR, Jamieson DJ, Powers AM, Honein MA. Zika virus. *N Engl J Med*. 2016;374(16):1552–1563. doi:10.1056/NEJMra1602113.
54. Samarasekera U, Triunfol M. Concern over Zika virus grips the world. *Lancet*. 2016;387(10018):521–524. doi:10.1016/S0140-6736(16)00257-9.
55. Cital Dogan A, Wayne S, Bauer S, et al. The Zika virus and pregnancy: evidence, management, and prevention. *J Matern Fetal Neonatal Med*. 2016;17: 1–11. doi:10.3109/14767058.2016.1174210.
56. Chan JFW, Choi GKY, Yip CCY, Cheng VCC, Yuen KY. Zika fever and congenital Zika syndrome: an unexpected emerging arboviral disease. *J Infect*. 2016;72(5):507–524. doi:10.1016/j.jinf.2016.02.011.
57. Waddell LA, Greig JD. Scoping review of the Zika virus literature. *PLoS One*. 2016;11(5):e0156376. doi:10.1371/journal.pone.0156376.
58. Zanluca C, Duarte dos Santos CN. Zika virus—an overview. *Microbes Infect*. 2016;18(5):295–301. doi:10.1016/j.micinf.2016.03.003.
59. Chen HL, Tang RB. Why Zika virus infection has become a public health concern? *J Chin Med Assoc*. 2016;79(4):174–178. doi:10.1016/j.jcma.2016.03.001.