

Gottesfeld–Hohler Memorial Foundation Zika Virus Think Tank Summary

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At a think tank bringing together experts on fetal neuroimaging, obstetric infectious diseases, and public health, we discussed trends in all of these areas for Zika virus. There is a wide variety of imaging findings in affected fetuses, influenced by timing of infection and probably host factors. The resources for diagnosis and interventions also vary by location with the hardest hit

areas often having the fewest resources. We identified potential areas for both research and clinical collaboration as the Zika virus epidemic continues to evolve.

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For a list of contributors, see Appendix 1, available online at <http://links.lww.com/AOG/B73>.

Each author has indicated that he or she has met the journal's requirements for authorship.

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In June 2017, the Gottesfeld-Hohler Foundation, a non-profit organization dedicated to ultrasound education, organized and sponsored a meeting in Fort Lauderdale, Florida, that involved a small working group of international investigators with special interest in the fetal central nervous system (CNS) along with representatives from several professional organizations. The countries represented in this forum were Brazil, Colombia, Haiti, Puerto Rico, and the United States. The organizations represented were The Eunice Kennedy Shriver National Institute of Child Health and Human Development, the Centers for Disease Control and Prevention (CDC), the American Institute of Ultrasound in Medicine (AIUM), the American College of Obstetricians and Gynecologists, the American College of Radiology, the Society of Radiologists in Ultrasound, the Society for Maternal-Fetal Medicine (SMFM), Federal University of Rio De Janeiro, and Instituto Professor Joaquim Amorim Neto. The goals were:

1. To better understand how the virus affects the fetal CNS
2. To discuss the best ways to identify fetal CNS injury in Zika virus–positive patients
3. To initiate data-sharing and collaborative activities.

This summary includes information presented at the meeting combined with data already published.

EPIDEMIOLOGY

The Zika virus is a single-stranded RNA virus. It was first identified in 1947, when it was isolated in rhesus



macaque monkeys in the Zika forest in Entebbe, Uganda.¹ Over the next decades, human cases of Zika virus occurred throughout Africa and Asia. In 2007, an outbreak occurred on Yap Island, Micronesia, during which approximately 75% of the population was infected.¹ Perinatal transmission was identified retrospectively as a result of the identification of microcephaly and cerebral malformations among children born after the infection of 66% of the population of French Polynesia in 2013.^{2,3} The extent of the fetal effect of Zika virus was not recognized until the virus spread to Brazil in 2014 where 1.3 million people subsequently have been infected with 14,000 fetuses or neonates suspected of having microcephaly and other congenital CNS infections and 2,775 neonates with microcephaly confirmed postnatally.⁴ In addition, vector-borne Zika virus is present in 84 countries, 31 of which have reported cases of Zika virus-related microcephaly or other CNS abnormalities.⁵

Mosquito-borne infections are seasonal and epidemics abate as more individuals become immune. This happened in Polynesia and now is evidenced by a major dropoff in Zika virus-related anomalies in Brazil. The peak season for Zika virus infection in Brazil in 2015 and 2016 had been during the winter and early spring. During the same time in 2017, less than 50 new cases of Zika virus congenital syndrome have emerged.⁴ In addition, the CDC has reported no new cases of mosquito transmission of Zika virus in the United States since December 2016.⁶ Nevertheless, many other areas of the world are still vulnerable, and according to an August 2017 CDC report, 203 new travel-related cases have appeared in the United States since January 1, 2017.⁶

CLINICAL BACKDROP

Most cases of Zika virus result from mosquito bites, predominantly by the *Aedes aegypti* mosquito,¹ although the virus can also be transmitted sexually or through other blood and body fluid exposures.⁷ The most common symptom of Zika virus infection is a maculopapular rash, sometimes accompanied by flu-like symptoms or conjunctivitis. Between 50 and 80% of Zika virus-positive individuals will not display clinical signs.

RNA polymerase chain reaction can be used for early identification of the virus in body fluids, although the duration of Zika virus detection after infection varies by body fluid. The virus generally has a relatively short lifespan in blood and urine with a median loss of Zika virus RNA detection at 8 and 14 days, respectively, but a recent cohort of 150 Zika virus-positive patients showed the 95th percentile of

time to loss of RNA was 54 days in serum, 39 days in urine, and 81 days in semen.⁸ If more than 14 days have passed since the possible infection, the diagnosis can be suspected by elevations in Zika virus immunoglobulin (Ig) M, but the accuracy is mitigated by IgM crossreactivity with other viruses such as Dengue and Chikungunya.⁹ Zika virus will invade the placenta, where it may act as a reservoir and a replication site for the virus. Here it often causes large areas of chorionic villus necrosis and massive fibrin deposition.¹⁰

FETAL CENTRAL NERVOUS SYSTEM EFFECTS

Investigators in Brazil¹¹ have found Zika virus to affect the fetal CNS in three ways:

1. A direct effect on the viability of neural progenitor cells resulting in calcifications in the gray-white junction, small brain volume, enlarged subarachnoid space, mild or moderate ventriculomegaly, and, eventually, microcephaly, which can be severe. A small head size is often associated with an abnormal skull shape, overlapping sutures, and redundant scalp skin. Other CNS findings are commonly present including dysgenesis of the corpus callosum, migrational abnormalities, and posterior fossa abnormalities.
2. Calcifications at the gray-white junction as the only finding, noted more commonly with exposure to the virus later in pregnancy.
3. Severe obstructive ventriculomegaly (aqueductal stenosis). The head circumference may be normal or enlarged.

The group's common opinion was that microcephaly, the dramatic finding that first alerted investigators to the CNS effects of Zika virus, represents the tip of the iceberg. Data presented at the meeting suggest that, in Puerto Rico, Brazil, and Colombia, more than 90% of affected fetuses displayed ventriculomegaly, calcifications in the gray-white matter junction, or a dysplastic corpus callosum. Less common findings were cortical migration abnormalities as well as those involving the posterior fossa such as vermian hypoplasia, mega cisterna magna, and, later, smaller than expected transcerebellar diameter measurements. By the time the diagnosis of microcephaly is made (head circumference greater than 3 SDs below the mean), there has already been brain parenchymal loss and an accompanying increase in subarachnoid fluid. In cases of first-trimester Zika virus infection, head circumference growth falls off by approximately 8–10 weeks, whereas in later infection, the growth slopes of other biometric measurements are compromised first, suggesting that late infection has a greater



initial effect on the placenta with resultant fetal growth restriction.

A spectrum from talipes equinovarus to arthrogryposis may represent CNS-related or motor neuron effects.¹² In infants, chorioretinal atrophy, optic nerve hypoplasia, and retinal pigmentation have been noted. Hearing loss can be a later manifestation of in utero infection.

For reasons that are unclear, the behavior of Zika virus varies among regions. For example, certain regions of Brazil appear to be outliers regarding the frequency of fetal CNS effects. A report from Rio de Janeiro showed that CNS damage occurred in 55%, 52%, and 29% with first-trimester, second-trimester, and third-trimester Zika infections, respectively.¹² In French Guiana, the trimester exposure frequency for CNS effects was 12.5%, 13.5%, and 3.2%.¹³ In Colombia and Puerto Rico, CNS abnormalities occurred in less than 5% of Zika virus-positive women over the course of pregnancy, with most resulting from first-trimester exposure. In the United States, where most cases are travel-related, data show an approximately 6–11% chance of a fetal CNS effect after Zika virus infection, most after first-trimester exposure.¹⁴

The time Zika virus takes to produce fetal CNS effects also varies, again for unclear reasons. For example, the shortest time from rash to recognized fetal CNS effects was 4 weeks in French Guiana¹⁴ and 8 weeks in Colombia.¹⁵ The median times from rash to microcephaly was 18 weeks in Colombia and 21 weeks according to pooled data from the literature.¹⁵ Thus far, in Puerto Rican data presented at the meeting, no CNS-affected fetuses with documented exposure to Zika virus in the first trimester developed clear CNS signs before 22 weeks of gestation.

Differences between countries may represent varying immune responses in vulnerable populations, synergistic interactions with other viruses such as Dengue,¹⁶ the quality and timing of ultrasound examinations, and a lack of similarity in data reporting and diagnostic definitions, including differences in definitions of microcephaly.

Regarding comparisons between Zika virus and cytomegalovirus, investigators in the assembled group found that features in common were parenchymal calcifications, ventriculomegaly, often with septations and cysts, small transcerebellar diameters, small cerebellar vermes, and large cisternae magna. However, the major difference relates to the location of the cerebral calcifications with cytomegalovirus having a predilection for the periventricular area and cortex, whereas Zika virus has been linked to larger calcifications located at the gray–white junction.

APPROACHES TO CENTRAL NERVOUS SYSTEM IMAGING IN ZIKA VIRUS-POSITIVE WOMEN

Unfortunately, clinicians attempting to make earlier and more precise diagnoses of CNS pathology in Zika virus-positive pregnant women face formidable challenges. There is inconsistent availability of resources, especially in Zika virus-endemic areas. Also, there have been difficulties in drafting guidelines for a disease whose CNS effects are still not fully understood. Nevertheless, the group agreed that strategically applied imaging can be effective in diagnosing most CNS abnormalities.

In areas without access to more sophisticated imaging resources, head circumference measurements (particularly after first-trimester exposure) and estimated fetal weight (especially after second-trimester exposure) from standard formulas can be compared with appropriate population-based nomograms. At that time, overt hydrocephalus and marked parenchymal calcifications can also be identified. In centers with ultrasound expertise, a thorough neurosonography examination¹⁷ can be undertaken using appropriate transabdominal and transvaginal approaches. This will enable the detection of the early, but often subtle, findings noted previously.

Magnetic resonance imaging can complement neurosonography by providing better evaluation of sometimes inaccessible midline structures such as the cerebellar vermis and brainstem as well as information regarding cortical sulci and gyri. This, in turn, may help with patient counseling¹⁸ and the timing of later recommended imaging.

EDUCATION

Without suitable expertise in sophisticated CNS imaging, diagnostic services may fall short of expectations. However, the workshop group felt that by providing more education, such as webinars as offered by AIUM, and making tools available to perform more extensive ultrasound examinations in limited resource settings, diagnostic accuracy may improve. Additionally, to aid clinicians in caring for Zika virus-exposed patients, AIUM, SMFM, the American College of Obstetricians and Gynecologists, and the International Society of Ultrasound in Obstetrics and Gynecology have published clinical guidelines¹⁹ and educational texts¹⁷ are available to help diagnose fetal CNS abnormalities.

FUTURE DIRECTIONS

Further Development of Diagnostic Tools

Improving the diagnosis of congenital Zika virus infection involves more sensitive and specific



maternal serologic testing. It is necessary to develop a specific IgG-based diagnostic test with limited crossreactivity to the related Dengue flavivirus in endemic areas. This and the development of an IgG avidity test will be key to stratifying the true risk of infection. Investigation into other diagnostic methods, utilizing unrefrigerated serum, saliva, or sperm, could enhance diagnostic capabilities in low-resource settings.

Ultrasound Characterization of Central Nervous System Pathology

Optimal ultrasound surveillance during pregnancy should be a prioritized area of research. It is not clear whether there are consistent pathways in ultrasound-definable pathology or if growth trajectories of affected fetuses are altered before or subsequent to neuroanatomic changes. The importance of using standard or local population-based growth nomograms should be prioritized, because often it is left to discretion of the health care provider.

Fetal Magnetic Resonance Imaging

Magnetic resonance imaging has been shown to identify more subtle changes in the fetal brain, but its role in a cogent diagnostic scheme needs further evaluation from a public health standpoint. When is it helpful and when is it essential? It simply will not be available in low-resource settings, but even in areas where magnetic resonance imaging is available, it is unclear whether the additional findings shown by magnetic resonance imaging will alter patient care. The value of this modality in the diagnosis of pathology and in the development of postnatal care plans requires further longitudinal investigation.

Continuum of Zika Virus Infection Beyond Birth

Understanding the pathogenesis of this disease will require correlation of prenatal ultrasound findings with longitudinal neonatal and pediatric imaging and neurodevelopment assessments. This type of investigation might need a multicenter approach and should involve collaboration with specialists in postnatal imaging and neonatal and pediatric neurology. Importantly, by focusing on the in utero and postnatal imaging findings that are the best predictors of long-term outcomes, early-encounter counseling and later neonatal management plans will be enhanced.

Perhaps the most fruitful information will emerge from a comprehensive study already in progress. The Zika in Infants and Pregnancy Study has been developed and supported by the *Eunice Kennedy Shriver*

National Institute of Child Health and Human Development, the National Institute of Allergy and Infectious Diseases, the National Institute of Environmental Health Sciences, and Brazil's Fundação Oswaldo Cruz. The comprehensive protocol was initiated to assess the number and character of fetal abnormalities with follow-up through early childhood. Secondary outcomes will include seroconversion rates, effect of coinfections, persistence and shedding of the virus, and long-term clinical sequelae. Thus far, 3,000 of the 10,000 patients envisioned have been enrolled.

Centers of Excellence

Dealing with the scope of knowledge needed to cope with advances in Zika virus scientific literature and the evolving understanding of Zika virus-induced fetal findings represents a formidable challenge for even the most diligent clinician. However, support can be available through the development of Centers for Excellence where images can be electronically received and interpreted by experts or consultation can be attained through teleconferencing. In addition to consultative services, house databanks can be established to store images and other information that can be used for collaborative research projects. This in no way would replace the training needed to maintain diagnostic sustainability in the outlying screening clinics.

Other Potential Areas of Research and Clinical Development

The working group members felt that nonimaging areas needing investigative attention were 1) the potential use of microparticle or cell-free fetal DNA technology as diagnostic tools; 2) the investigation of maternal inflammatory cytokine profiles and methylation patterns for both diagnosis and prognosis; 3) the further characterization of Zika virus-induced placental abnormalities and their relation, if any, to growth trajectories and birth outcomes; and 4) epidemiologic studies to identify the high-risk population and generate a risk score for potentially affected fetuses in areas where disease is endemic and testing is not widely available.

Ongoing Research With Potential Therapeutic and Preventive Effect

In the next year ongoing studies, not directly targeting the fetal CNS, should yield important and, hopefully, encouraging information regarding the treatment and prevention of Zika virus infection. Brazilian investigators have noted subjective improvement in a few pregnancies in which the pregnant woman was treated



with the antimalarial chloroquine.²⁰ An antiviral medication (sofosbuvir), used successfully in hepatitis C, has had promising results in mice infected with the virus.²¹ Also, in Brazil, early intervention measures are being studied in children infected by the Zika virus in utero. Last, attenuated virus and nucleoside-modified RNA vaccines have been developed, which have shown promise in providing immunity²² and protection against congenital effects of Zika virus infection²³ in animal models. Human testing is in progress. When available, this could have a significant positive effect, especially if offered at a reasonable cost.

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