What is a Teratogen?

- An exposure in pregnancy that has a harmful effect on the embryo or fetus
- Types of teratogens:
  - Medications
  - Heavy metals
  - Radiation
  - Maternal conditions
  - Infections
  - Procedures
  - Other

History of Zika Virus and Microcephaly

- 1947: Zika virus identified in macaque in Uganda (Zika Forest)
- 1953: Zika virus recognized as cause of human illness in Nigeria
- 2007: Large outbreak of Zika virus illness in the State of Yap, Federated States of Micronesia
- 2012-2014: Large outbreak of Zika virus illness in French Polynesia
- March 2015: Zika virus first identified in the Americas in Brazil
- Sept 2015: Increased number of infants born with microcephaly noted in Brazil
- Early 2016: Increase in microcephaly retrospectively noted in French Polynesia following the 2013-2014 outbreak
- Jan 2016: CDC issues interim travel guidance for pregnant women for areas with ongoing Zika virus transmission
**CDC Lab Confirms Zika In Fetal Tissues**

- Zika virus shown to be present in fetal tissue
- Evidence of Zika virus has been detected in
  - Amniotic fluid
  - Placenta
  - Fetal brain tissue
  - Products of conception

---

**Challenges to Determining Teratogenicity of Zika Virus (early 2016)**

- Large proportion of people infected with Zika infection asymptomatic
- Laboratory testing initially not widely available (most early cases not laboratory-confirmed) and IgM testing challenging (cross-reactivity with other flaviviruses, length of IgM persistence unknown, etc.)
- Consistent and standardized case definitions of microcephaly not being used and baseline rate of microcephaly not well defined
- Mosquito-borne viruses not previously recognized as teratogenic in humans
- Rumors circulating about other possible causes (e.g., insecticides, genetically modified mosquitoes, vaccines)

---

**Does Zika Virus Cause Adverse Pregnancy Outcomes?**

- Koch’s Postulates
- Bradford Hill Criteria
- Shepard's Criteria for Teratogenicity
Koch’s Postulates

- [Micro]Organisms must be consistently detected in the locally affected tissues [...]
- The organisms [that seem plausible candidates for pathogens] must be isolated and grown in pure culture
- It must be possible to reproduce the disease using those pure cultures

Bradford Hill Criteria

- Temporal relationship
- Strength
- Dose-Response Relationship
- Consistency
- Plausibility
- Consideration of Alternate Explanations
- Experiment
- Specificity
- Coherence

How Have Previous Teratogens Been Identified?

Two Approaches

- “Astute clinician” approach – rare exposure-rare defect (most teratogens)
- Use of epidemiologic data to confirm an association
Shepard’s Criteria for Teratogenicity

Shepard’s Criteria for Teratogenicity (continued)

Shepard’s Criteria for Teratogenicity

- Shepard Criterion #1 – Proven exposure to agent at critical time(s) in prenatal development
  - Yes, timing for severe microcephaly and intracranial calcifications appears to be late 1st/early 2nd trimester, based on information on some confirmed and many presumed cases of Zika virus infection
Shepard’s Criteria for Teratogenicity

- Shepard Criterion #2 – Consistent findings by two or more epidemiologic studies of high quality
  - Control of confounding factors
  - Sufficient numbers
  - Exclusion of positive and negative bias factors
  - Prospective studies, if possible
  - Relative risk of 6 or more
  - Most data at that time were case series, not epidemiologic studies
  - Two epidemiologic studies that examined this association: Brasil et al. and Cauchemez et al.

Zika Virus and Microcephaly

- Study in Brazil: 42 women with laboratory-confirmed Zika virus infection with prenatal ultrasound
  - 12 (29%) abnormalities detected, including 2 intrauterine fetal deaths
- 2013-14 outbreak in French Polynesia
  - 8 cases of microcephaly identified
  - Modeling estimated infection with Zika during 1st trimester of pregnancy resulted in microcephaly risk of ≈1%

Shepard’s Criteria for Teratogenicity

- Shepard Criterion #3 – Careful delineation of clinical cases. A specific defect or syndrome, if present, is very helpful.
  - Phenotype in cases with presumed congenital Zika virus infection – brain abnormalities, including microcephaly and intracranial calcifications, cutis verticis gyrata, eye findings, arthrogryposis, club foot, IUGR, etc. – some authors have used the term “presumed congenital Zika syndrome”
  - Findings in some cases consistent with fetal brain disruption sequence

Schuler-Faccini et al., MMWR Morb Mortal Wkly Rep 2016; 65(3):19-22
Costa et al., Ann Intern Med 2016; 164(10):688-91
Miranda-Filho et al., Am J Public Health 2016; 106(4):598-600
Shepard’s Criteria for Teratogenicity

- Shepard Criterion #4 – Rare environmental exposure associated with rare defect
  - Rare defect
    - Congenital microcephaly – birth prevalence in US - 2-12/10,000
    - Fetal brain disruption sequence – no data on birth prevalence, but rare
  - Zika virus infection - “rare exposure”?
  - Zika virus infection – rare exposure among travelers

Brain Abnormalities in Fetus Born to Traveler and Prolonged Viremia

Case report
- Pregnant woman traveled during 13th week of gestation to Mexico, Guatemala, and Belize (Nov 22-29); symptom onset at 12 weeks gestation
- Prenatal ultrasound
  - Decrease in head circumference from 47th percentile at 16 weeks to 24th percentile at 20 weeks
  - Abnormal intracranial anatomy at 19 weeks
  - Fetal MRI at 20 weeks: brain abnormalities, including diffuse cerebral atrophy
- Postmortem evaluation following pregnancy termination at 21 weeks
  - Diffuse cerebral cortical thinning
  - High levels of Zika virus RNA; virus was cultured from brain tissue

Zika Virus and Fetal Brain Disruption Sequence

- Findings in some cases were consistent with fetal brain disruption sequence
- First described in 1984 but noted in earlier literature
- Fetal brain disruption sequence includes severe microcephaly, overlapping sutures, prominent occipital bone, scalp rugae, and marked neurological impairment
Shepard’s Criteria for Teratogenicity

- Shepard Criterion #5 — Teratogenicity in experimental animals important, but not essential
  - No animal model published with Zika virus infection during pregnancy and fetal effects

- Shepard Criterion #6 — The association should make biologic sense
  - Consistent with findings in other viral teratogens
  - Pathologic findings implicate Zika virus is neurotropic and damages brain tissue
  - Other flaviviruses are teratogenic in animals
Shepard’s Criteria for Teratogenicity

- Shepard Criterion #7 – Proof in an experimental system that the agent acts in an unaltered state. Important information for prevention
  - Applies to medications, not to infectious causes

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Does Zika Virus Cause Adverse Pregnancy and Birth Outcomes?

Criteria for Proof of Human Teratogenicity

<table>
<thead>
<tr>
<th>Criterion</th>
<th>Criterion Met?</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Proven exposure to agent at critical time(s) during prenatal development</td>
<td>Yes</td>
</tr>
<tr>
<td>2. Consistent findings by ≥2 high-quality epidemiologic studies</td>
<td>Partially</td>
</tr>
<tr>
<td>3. Careful delineation of clinical cases</td>
<td>Yes</td>
</tr>
<tr>
<td>4. Rare environment exposure associated with rare defect</td>
<td>Yes</td>
</tr>
<tr>
<td>5. Teratogenicity in experimental animals important but not essential</td>
<td>No</td>
</tr>
<tr>
<td>6. Association should make biologic sense</td>
<td>Yes</td>
</tr>
<tr>
<td>7. Proof in an experimental system that the agent acts in an unaltered state</td>
<td>NA</td>
</tr>
</tbody>
</table>

Zika Is a Cause of Microcephaly
(Released by NEJM on April 13, 2016)
“Never before in history has there been a situation where a bite from a mosquito could result in a devastating malformation.”
– Dr. Tom Frieden, former CDC Director, April 13, 2016

“...the last time an infectious pathogen (rubella virus) caused an epidemic of congenital defects was more than 50 years ago...”
– New England Journal of Medicine, April 13, 2016

Discussion

- Identification of Zika virus as a human teratogen - too soon or too late?
- Do Shepard's criteria need updating?
- Additional data accumulated since publication of N Engl J Med paper that support teratogenicity
  - Animal models, including mice (Cugola et al., 2016; Li et al., 2016; Miner et al., 2016) and chick (Goodfellow et al., 2016) models
  - Registry data from US and territories (Honein et al., 2017; Reynolds et al., 2017; Shapiro-Mendoza et al., 2017)
  - Epidemiologic data, including case-control study with overall odds ratio of 55.5 (95% CI, 8.6-infinity) (de Araujo et al., 2016)

Thank you!
Sonja Rasmussen, MD, MS
skr9@cdc.gov

For more information, contact CDC
1-800-CDC-INFO (232-4636)
TTY: 1-888-232-6468
www.cdc.gov

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.
Congenital Infections

Exploring Their Role in Birth Defects and Other Adverse Pregnancy Outcomes

Cynthia A. Moore, MD, PhD
2017 National Birth Defects Prevention Network Annual Meeting
September 13, 2017

Congenital Infections

- Infections vertically transmitted from a pregnant woman to her embryo or fetus during pregnancy
- Variable effects depending on type of pathogen, timing during pregnancy, maternal and other factors – and information is limited on many pathogens
- Clinical manifestations also vary (e.g., asymptomatic, mild, non-specific)
- Information greatest on congenital infections encompassed under various iterations of the TORCH acronym
- Emerging infections often lack information on effects during pregnancy
  - New or caused by genetically changed existing organisms
  - Geographic spread of known pathogens to new populations
  - Previously unrecognized infections that emerge because of changes in the ecology
  - Previously controlled infections that have re-emerged (e.g., antimicrobial resistance)


Congenital Infections and Birth Defects

Known Teratogens

- Toxoplasma gondii
- Treponema pallidum
- Rubella
- Cytomegalovirus
- Herpesvirus 1 & 2
- Varicella-zoster virus
- Lymphocytic choriomeningitis virus
- Parvovirus B19
- Zika virus
Understanding and Responding to Congenital Infections

• Natural history (life cycle) of the pathogen and transmission to human host
• Mechanisms of vertical transmission
• Embryonic/fetal effects (pathogenesis and influencing factors)
• Diagnostic and treatment methods
• Epidemiology
• Prevention and control

The Placental Barrier

• Syncytiotrophoblast barrier occurs by the end of implantation (~7 days)
• Uteroplacental circulation established and placenta sole barrier (~12 weeks)
  • Mechanisms differ early vs late pregnancy
• Mechanisms of vertical transmission
  — Direct or contiguous infection of cell layers (e.g., maternal vascular endothelial cells, extravillous trophoblasts)
  — Trafficking of infected maternal immune cells across the placental barrier
  — Cell-associated transport from maternal blood to fetal capillaries
  — Breach secondary to syncytiotrophoblast layer or villus tree damage
  — Transvaginal ascending infection

Overcoming the Placental Barrier

Figure 1. Routes used by TORCH pathogens to overcome the placental barrier. Reprinted by permission from Macmillan Publishers Ltd: Nature Review Microbiology, 2016 Nov;14(11):717-725.
Types of Birth Defects

- Results from an intrinsically abnormal developmental process - neural tube defect
- Results from an extrinsic breakdown of an originally normal developmental process - amniotic band sequence
- Results from an abnormal organization of undifferentiated tissue - achondroplasia

Adapted from Goodman & Gorlin, The Malformed Infant and Child, Oxford University Press, 1983

Congenital Infection and Clinical Phenotypes

- Early pregnancy loss
- Infants with a disruption birth defect
- Other adverse pregnancy outcomes
- Birth with developmental disabilities alone

Congenital Infection and Clinical Phenotypes

<table>
<thead>
<tr>
<th>Congenital Phenotype</th>
<th>Rubella</th>
<th>Congenital CMV</th>
<th>Toxoplasmosis</th>
<th>LCMV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CNS development</td>
<td>Fetal death</td>
<td>Fetal death</td>
<td>Fetal death</td>
<td>Fetal death</td>
</tr>
<tr>
<td>Birth defects - Head &amp; neck</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Birth defects - Cardiac</td>
<td>Transient cardiac failure</td>
<td>Transient cardiac failure</td>
<td>Transient cardiac failure</td>
<td>Transient cardiac failure</td>
</tr>
<tr>
<td>CNS development</td>
<td>Microcephaly</td>
<td>Microcephaly</td>
<td>Microcephaly</td>
<td>Microcephaly</td>
</tr>
<tr>
<td>Birth defects - Respiratory</td>
<td>Hypoplasia of trachea and lungs, bronchiectasis</td>
<td>Hypoplasia of trachea and lungs, bronchiectasis</td>
<td>Hypoplasia of trachea and lungs, bronchiectasis</td>
<td>Hypoplasia of trachea and lungs, bronchiectasis</td>
</tr>
<tr>
<td>Other systemic &amp; sensory disorders</td>
<td>Prolactin deficiency</td>
<td>Prolactin deficiency</td>
<td>Prolactin deficiency</td>
<td>Prolactin deficiency</td>
</tr>
<tr>
<td>Other adverse pregnancy outcomes</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>None</td>
</tr>
</tbody>
</table>
Zika Virus (ZIKV) – Newly Identified Teratogen

• Single stranded RNA virus
• Closely related to dengue, yellow fever, Japanese encephalitis, and West Nile viruses
• Primarily transmitted by the Aedes aegypti mosquito
• Additional modes of transmission
  – Intratuterine and perinatal transmission
  – Sexual transmission
  – Laboratory exposure
  – Probable blood transfusion, organ transplantation
  – Possible breast milk

History of Zika Virus and Link to Microcephaly

• 1947 Zika virus identified in macaque in Uganda (Zika Forest)
• 1953 Zika virus recognized as cause of human illness in Nigeria
• 2007 Large outbreak of Zika virus illness in the State of Yap, Micronesia
• 2013 - 2014 Large outbreak of Zika virus infection in French Polynesia
• Mar 2015 Zika virus first identified in the Americas in Brazil
• Sept 2015 Increased number of infants born with microcephaly noted in Brazil
• Early 2016 Increase in microcephaly retrospectively noted in French Polynesia following the 2013 - 2014 outbreak
• Jan 2016 CDC issues interim travel guidance for pregnant women for areas with ongoing Zika virus transmission
• Feb 2016 WHO declared Public Health Emergency of International Concern (PHEIC)
• Nov 2016 WHO declared Zika a significant enduring public health challenge requiring intense action but no longer represents a PHEIC
• Mar 2017 Affected infants continue to be born in the Americas and beyond

Zika Virus in Pregnancy

• Incidence of Zika virus infection in pregnant women is not known
• Infection can occur in any trimester
• No evidence of more severe disease compared with non-pregnant women
• No evidence of increased susceptibility during pregnancy
• Pregnant women can be infected
  • Through a mosquito bite
  • Through sex without a condom with an infected partner
• Zika virus can be passed to the fetus during pregnancy or around the time of birth
**Congenital Zika Syndrome Cranial Morphology**

- Severe microcephaly (most more than 3 SD below the mean)
  - Partial collapse of the skull with overlapping sutures
  - Occipital bone prominence
  - Small or absent anterior fontanel
  - Scalp rugae
- Consistent with fetal brain disruption sequence (FBDS)
- Not all with severe microcephaly have FBDS phenotype
- FBDS is rare but not unique to congenital Zika syndrome

---

**Congenital Zika Syndrome – In Utero Cranial Morphology**

- Thin cerebral cortex
- Intracranial calcifications primarily subcortical in location
- Hydrocephalus and hydrocephalus ex-vacuo
- Hydranencephaly
- Gyral abnormalities (polymicrogyria by imaging)
- Absent or hypoplastic corpus callosum
- Hypoplasia of the cerebellum or cerebellar vermis

*extremely rare or unique finding*
Congenital Zika Syndrome

Ocular Findings

- Structural and anterior eye anomalies
  - Microphthalmia, coloboma
  - Cataracts, intraocular calcifications
- Posterior eye anomalies
  - Optic nerve hypoplasia, atrophy
  - Choriretinal atrophy and scarring*
  - Macular pallor*
- Gross pigmentary anomalies – generally in the macular area*
- No active choriorretinitis yet reported
*extremely rare or unique finding

Congenital Zika Syndrome

Contractures

- Isolated and multiple congenital contractures (arthrogryposis) reported
- Clinical picture varies (e.g., large and small joints, upper or lower limbs or both, amoplasia phenotype)
- Not previously associated with fetal brain disruption sequence phenotype*
- Reported but not well-documented with other congenital infections (e.g., rubella, varicella)
- Report of spinal cord hypoplasia in infants with contractures
*extremely rare or unique finding

Congenital Zika Syndrome – Neurologic Sequelae

- Information on long-term medical and developmental outcomes or mortality sparse
- Neurologic sequelae reported to date in addition to contractures include the following:
  - Motor and cognitive disabilities (French Polynesia)
  - Epilepsy
  - Swallowing difficulties
  - Irritability with excessive crying
  - Vision loss and hearing impairment
  - Hypertonia and spasticity with tremors (pyramidal symptoms)
  - Dystonia and dyskinesia (extrapyramidal symptoms)*
  - Paralysis of the diaphragm
*extremely rare or unique finding
Congenital Zika Syndrome – Components of Unique Pattern

- Severe microcephaly with partial skull collapse
- Intracranial calcifications in the subcortical region
- Macular scarring and focal pigmentary retinal mottling
- Congenital contractures
- Neurologic abnormalities both pyramidal and extrapyramidal

Prenatal Zika Virus Infection – Congenital Zika Syndrome

ZIKA VIRUS Disrupts future developmental processes and Destroys existing neural tissue

Loss of brain volume
- Severe microcephaly
- Misshapen skull w/ overlapping sutures
- Redundant scalp

Loss of brain volume
- Severe microcephaly
- Misshapen skull w/ overlapping sutures
- Redundant scalp
Prenatal Zika Virus Infection – Congenital Zika Syndrome

ZIKA VIRUS
Disrupts future developmental processes and Destroys existing neural tissue

Loss of brain volume

Neurologic dysfunction

- Severe microcephaly
- Misshapen skull w/ overlapping sutures
- Redundant scalp

Hearing, vision, swallowing problems
- Global developmental impairment
- Limb contractures
- Hypertonia, hypotonia, epilepsy, extreme irritability, dystonia, dyskinesia

Recognizable pattern = congenital Zika syndrome

Congenital Zika Syndrome – Expanding the Phenotype

- Possible expansion to infants with
  - Brain or eye anomalies but no microcephaly
  - Sensory/cranial nerve dysfunction with delayed onset
  - Other CNS anomalies with origin in the embryonic period
  - Non-neurologic congenital anomalies
  - Growth restriction
Congenital Zika Syndrome –
Longer Term Medical Sequelae

• Longer term sequelae reported to date include the following:
  ▪ Motor and cognitive disabilities (French Polynesia)
  ▪ Hydrocephaly – some requiring a VP shunt
  ▪ EEG abnormalities in infants without clinical seizures; worsening epilepsy
  ▪ Feeding problems and severe reflux – some requiring a g-tube
  ▪ Respiratory problems – diaphragmatic paralysis
  ▪ Glaucoma
  ▪ Potential cerebral palsy
  ▪ Potential endocrine abnormalities
  ▪ Microcephaly onset after birth

Congenital Zika Virus Infection
Many Questions Remain – Some Partially Answered

• What is the full range of potential reproductive health problems that Zika virus infection may cause?
• Are there differences between Asian and African strains in terms of teratogenic potential and if so what underlies the differences?
• How long does the virus persist in various tissues after infection?
• What is the level of risk from a Zika virus infection during pregnancy?
• When during pregnancy does Zika virus infection pose the highest risk to the fetus?
• What are other factors (e.g., preceding or co-occurring infection, nutrition, presence of symptoms) that might affect the risk for birth defects?
• What are the expected patterns of anomalies comprising congenital Zika syndrome?

Studying the Impact of Emerging Infections on Embryo or Fetus – Challenges

• Effects of infection on the fetus are unknown and difficult to predict
  ▪ Vary depending on infectious agent and timing of infection
  ▪ Include spontaneous abortions, preterm birth, intrauterine growth restriction, birth defects and developmental disabilities
  ▪ Later manifestations such as cognitive impairment might occur in infants appearing normal at birth

Infection could be mild or asymptomatic among pregnant women, despite significant effects on the embryo or fetus. Infections might be missed because appropriate diagnostic tests not done. Effects on fetus might differ depending on maternal severity and nature of illness, even in the absence of congenital infection.

- Diagnosis of infection in the fetus or infant can be challenging
  - Application of assays developed for adults to congenital infection can be difficult
  - Detection of specific IgM in infant serum provides strong evidence of infection, but false positives and false negatives occur
  - Sensitivity of diagnostic assays for newly-recognized teratogens might be unknown

Prophylaxis and treatment recommended for the general population might not be appropriate for pregnant women. Certain vaccinations or medications are contraindicated during pregnancy because of potential fetal effects. Fetal effects of most medications are not known. Might be difficult to separate effects of infection from effects of treatment.
Challenges in Determining Teratogenicity of Zika Virus (Early 2016)

- Large proportion of people infected with Zika infection asymptomatic
- Laboratory testing initially not widely available (most early cases not laboratory-confirmed) and IgM testing challenging (cross-reactivity with other flaviviruses, length of IgM persistence unknown, etc.)
- Consistent and standardized case definitions of microcephaly not being used and baseline rate of microcephaly not well-defined
- Mosquito-borne viruses not previously recognized as teratogenic in humans
- Rumors circulating about other possible causes (e.g., insecticides, genetically modified mosquitoes, vaccines)

Examples of Studies Used to Examine Fetal Effects of West Nile and Zika Virus – Rasmussen et al., 2017

<table>
<thead>
<tr>
<th>Type of Study Design</th>
<th>West Nile Virus</th>
<th>Zika Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case Report/series</td>
<td>Case reports of infected born to WNV-infected mothers (1) with congenital anomalies (4 infants)</td>
<td>Case reports of infants assessed because of microcephaly demonstrated a consistent phenomenon — congenital Zika syndrome</td>
</tr>
<tr>
<td>Birth defects surveillance system</td>
<td>Not used</td>
<td>CDC funded Rasmussen to conduct surveillance for birth defects linked to infection associated with congenital WNV infection</td>
</tr>
<tr>
<td>Pregnancy Registers</td>
<td>WHO Pregnancy Registry showed most laboratory infected mothers had no abnormalities at birth or at 1 year; follow-up at 5 years for growth, development; eye outcomes showed no evidence of abnormalities up to age 5 years</td>
<td>United States Zika Pregnancy Registry Zika Active Pregnancy surveillance system; USPWR data used to study prolonged virologic, symptomatic sequelae and estimate the risk of Zika-associated defects in utero; infants born to women possible infected with WNV during pregnancy</td>
</tr>
</tbody>
</table>

Examples of Studies Used to Examine Fetal Effects of West Nile and Zika Virus – Rasmussen et al., 2017

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<th>Zika Virus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cohort Studies</td>
<td>Prospective cohort study used to study the effects of WNV during pregnancy including extension of developmental abnormalities and birth defects, but long-term follow up needed</td>
<td>Prospective cohort of women with WNV during pregnancy who tested positive and were noted in surveillance to be at risk of Zika infection and, as such, had follow-up to determine if Zika exposed pregnant women are at an increased risk of Zika-affected pregnancies or 1% of Zika-infected pregnancies</td>
</tr>
</tbody>
</table>
| Case Control Studies | Not used | Preliminary results from case-control study conducted in Brazil demonstrated substantial association between congenital WNV infection and microcephaly with cranial OP SS, & a =
Prenatal Diagnosis and Imaging: CNS Abnormalities & Congenital Infections

CAREY EPPES, MD
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CHIEF OF OBSTETRICS, TOBAC HOSPITAL

MARTINA BADELL, MD
EMORY UNIVERSITY
DIVISION OF MATERNAL FETAL MEDICINE
DIRECTOR, EMORY UNIVERSITY HOSPITAL
MIDTOWN PERINATAL CENTER

Outline

- Prenatal diagnostic modalities for congenital infections
  - Imaging
    - Ultrasound
    - MRI
  - Maternal serology
  - Amniocentesis

- Congenital infections with associated CNS abnormalities
  - Zika
  - CMV
  - Toxoplasmosis

Ultrasonography in Pregnancy: Prenatal diagnosis

- Settings for prenatal ultrasound (U/S)
  - Ob/gyn office
  - Emergency Room
  - Perinatal Center/Maternal Fetal Medicine

- Types of prenatal ultrasound (U/S)
  - 2D
    - First trimester, anatomy, growth
  - 3D
    - Second trimester, anatomy, growth
MRI in pregnancy: Prenatal Diagnosis

- The principal advantage of MRI is the ability to image deep soft tissue structures
  - Not operator dependent
  - Does not use ionizing radiation
  - No precautions or contraindications specific to the pregnant woman

Utility

- Improved visualization of
  - Cerebral biometry, gyral/sulcal patterns, Cerebral parenchyma
- Demonstrated that fetal MRI yielded additional information compared to US and the ultrasound-based diagnosis and management were modified in 32% and 19% of cases, respectively (n = 145)

- Fetal MRI provides superior diagnostic capability compared to US concerning the fetal brain
- Access/Referral

Maternal serum testing for infection

- Indications for testing:
  - Maternal symptoms
  - Maternal exposure
  - Abnormal fetal ultrasound findings

- Types of testing:
  - IgM/IgG
  - NAT

Fetal Testing: Amniocentesis

- Amniocentesis Procedure:
  - Under ultrasound guidance, a needle inserted into amniotic sac to obtain small amount of amniotic fluid
  - Pregnancy related risk: 1/300-1/600

- Indications:
  - Genetic diagnosis
  - Infections
  - Other: lung maturity, Rh disease, polyhydramnios
Congenital infections with associated CNS abnormalities

- Zika
- Cytomegalovirus (CMV)
- Toxoplasmosis

Zika Virus

- Zika emerging mosquito borne flavivirus
- One of the rare infections in pregnant women → congenital anomalies
- Zika virus biology not fully defined → challenges managing & counseling about exposures and infection in pregnancy
- Maternal symptoms:
  - Most won’t have symptoms or will only have mild symptoms
  - The most common symptoms of Zika are
    - Fever
    - Rash
    - Headache
    - Joint pain
    - Conjunctivitis (red eyes)
    - Muscle pain

Zika: Screening & Diagnosis

- All pregnant women in US should be asked about possible Zika virus exposure before and during the current pregnancy at every prenatal visit
- Pregnant women with possible Zika exposure and symptoms should be tested to diagnose cause of their symptoms
  - NAT and IgM
- Asymptomatic pregnant women with ongoing possible Zika exposure should be offered Zika NAT testing 3 times during pregnancy
- Asymptomatic pregnant women with recent possible Zika exposure but without ongoing exposure not recommended to have Zika testing
- Pregnant women with possible Zika exposure who have a fetus with US findings concerning for Zika should be tested
  - NAT and IgM

MMWR July 24th, 2017
Zika: Diagnosis

- Serology test results can be difficult to interpret & adverse outcomes caused by Zika during pregnancy are not fully described.
  - Pregnant women with lab evidence of recent flavivirus infection are considered to have possible Zika virus infection & should be monitored frequently.
  - More ultrasounds

- Referral to a maternal-fetal medicine (MFM)/pediatrician or infectious disease specialist with expertise in pregnancy management is recommended.
  - Maternal infection or abnormal ultrasound


Congenital Zika Syndrome

- Pattern of birth defects found among fetuses/babies infected with Zika during pregnancy.

  5 features:
  - Severe microcephaly where the skull has partially collapsed.
  - Decreased brain tissue with a specific pattern of brain damage.
  - Damage to the back of the eye.
  - Joints with limited range of motion, such as clubfoot.
  - Too much muscle tone restricting body movement soon after birth.

- Some infants with congenital Zika virus infection who do not have microcephaly at birth may later experience slowed head growth and develop postnatal microcephaly.


Zika: Fetal Evaluation

- Ultrasound particularly if obtained close to the time of infection, may not preclude later manifestations.
  - Some reported only postnatal abnormalities.

- Some data suggested most severe adverse outcomes appear to be more common but are not limited to women infected in the first trimester.

- Data suggest that severe outcomes are not limited to symptomatic pregnant women.

- Repeat imaging should be considered if Zika testing suggests infection.

Fetal Evaluation via U/S

- Prenatal U/S to evaluate for fetal abnormalities consistent with congenital Zika virus syndrome is recommended for all pregnant women tested for Zika, regardless of laboratory findings.
- U/S to assess fetal anatomy (neuroanatomy) and fetal growth.
- They should look for development of findings such as:
  - Microcephaly
  - Hydrocephaly
  - Ventriculomegaly
  - Arthrogryposis
  - Abnormalities of the corpus callosum
  - Cerebrum
  - Cerebellum
  - Eyes
  - Other brain abnormalities


Zika - Microcephaly

- Head circumference
  - Less than 3 SD below the mean.

Zika: Microcephaly

![Ultrasound images of fetal head]
Zika: Ventriculomegaly

**Normal**

**Ventriculomegaly**
- Mild: >1.0-1.2 cm
- Moderate: >1.2-1.5 cm
- Severe: >1.5 cm

Zika: Intracranial calcifications

Zika: Corpus callosum abnormalities

- Corpus callosum is the largest midline commissure of the brain connecting the neocortex of the cerebral hemispheres.
- In complete agenesis of the corpus callosum (ACC), there is a total failure of the commissure to develop.

Incidence:
- Among live births: ~1/10,000
- Developmental disabilities: 2/100
- Existing CNS anomaly: ~47/100
- Associated with a broad range of clinical manifestations ranging from normal to severe psychomotor delay.
Zika Fetal Testing Amniocentesis?

- If US suspicion for fetal anomaly amniocentesis for Zika virus testing may be considered particularly if being performed for genetic testing

- Unknowns:
  - How long after a pregnant woman becomes infected she can transmit the virus to the fetus
  - Duration amniotic fluid will be ZIKV RNA NAT positive
  - Ability of the test to determine the presence of fetal injury

Zika: Newborn/Infant

- All infants born to mothers who have lab evidence of Zika infection during pregnancy should receive:
  - Comprehensive physical exam
  - Neurologic assessment
  - Head ultrasound
  - Standard newborn hearing assessment
  - Zika viremia testing

- Testing is recommended for infants:
  - Born to mothers who have laboratory evidence of Zika virus infection
  - Infants to have abnormal ultrasound suggestive of congenital Zika syndrome and a maternal epidemiologic link suggesting possible exposure during pregnancy, regardless of maternal test results.
  - Zika virus NAT test should be performed on both infant serum and urine

Cytomegalovirus (CMV)

- Epidemiology
  - Seroprevalence increases with age
    - Overall in US 59%
    - 1-4% of pregnant women are covert
  - Lower SES more likely to have been exposed
  - Day Care, health care workers

- Primary
  - Ongoing viral viremia can occur for 6 months

- Recurrent
  - Periodic virus shedding
CMV: Maternal Symptoms

- Asymptomatic, malaise, fever, generalized lymphadenopathy, and hepatosplenomegaly
- Patients who are immunocompromised may develop extremely serious sequelae of infection, including chorioretinitis and pneumonitis

CMV: Screening and Diagnosis

- IgG
- IgM
- May remain 9 to 12 months after infection
- May become positive in re-infection, or remain negative
- Avidity
- New infection: Low avidity
- Reactivation
- Amniocentesis PCR
- Interval from infection to amnio (7 weeks)
- Neonatal urine and blood PCR

CMV: Transmission

- Rates of transmission correlated with gestational age
- However severity is the inverse
- Transmission:
  - First trimester: 35%
  - Second trimester: 42%
  - Third trimester: 59%
- Severity (impact by termination rates)
  - First<Second<Third

Feldman et al. AJOG 2011
CMV: Diagnosis

- The most sensitive and specific test for diagnosing congenital CMV infection is the identification of CMV in amniotic fluid by either culture or PCR.
- Identification of the virus in amniotic fluid by culture or PCR does not necessarily delineate the severity of fetal injury.

CMV: Ultrasound images

- CNS:
  - Microcephaly
  - Ventriculomegaly
  - Brain Calcifications
- Echogenic bowel
- Growth restriction
- Calcifications: liver, spleen
- Hydrops

CMV: Ultrasound
CMV: Neonatal Findings

- Hepatosplenomegaly
- Thrombocytopenia with resultant petechiae
- Intracranial calcifications
- Small for Gestational Age

- Hepatitis and jaundice
- Microcephaly
- Chorioretinitis
- Hearing loss
- Mental retardation
- Seizures

Neonatal Outcomes: Primary CMV Infection

Exposed (Maternal Illness)

Infected (Evidence of Fetal Infection)

Affected at Birth (Congenital Infection)

Un-Affected at Birth

Late Infection

30-50%
5-15%
10-15%
0-2%
0-2%
5-10%

Neonatal Outcomes: Recurrent CMV Infection

Exposed (Maternal Illness)

Infected (Evidence of Fetal Infection)

Affected at Birth (Congenital Infection)

Un-Affected at Birth

Late Infection

10%
5-10%
Toxoplasmosis

- Maternal Infection: starts with ingestion of cysts from uncooked/undercooked meat of infected animals or contact with oocysts from infected cats or contaminated soil

- Fetal/neonatal disease:
  - More severe if maternal infection in 1st trimester
  - Incidence of transmission highest in 3rd trimester

- Prevention:
  - Avoid exposure
  - Cat litter
  - Undercooked meats
  - Washing fruits and vegetables

Toxoplasmosis: Symptoms Infected Pregnant Women

- Typically asymptomatic
- Mononucleosis-like symptoms
- Adenopathy
- Can be severe in immunosuppressed
  - Chorioretinitis
  - Brain abscess
**TOXO: Detection**

- (#1) IgM
  - Sensitivity 28%
  - Occasionally not detected in early infection
  - Can persist for months to years
  - Often negative in immunosuppressed

- (#2 a) Avidity
  - If IgM positive, this is next step

- (#2 b) IgG
  - Done if avidity not available
  - Repeat titer 3 weeks later
  - PCR
  - Amniocentesis or Cordocentesis

**Toxoplasmosis Testing**

<table>
<thead>
<tr>
<th>Antibodies</th>
<th>Uninfected</th>
<th>Recent/Acute infection</th>
<th>Chronic/latent infection</th>
</tr>
</thead>
<tbody>
<tr>
<td>IgM</td>
<td>Absent**</td>
<td>Present in almost all cases. Weeks - months</td>
<td>Most often absent, in 5% persisted for years**</td>
</tr>
<tr>
<td>IgG</td>
<td>Absent</td>
<td>Present. Range from low (2IU/mL) to high (300-6000IU/mL); titer Takes 3-6 months to reach peak</td>
<td>Present</td>
</tr>
<tr>
<td>IgG Avidity</td>
<td>Absent</td>
<td>Low</td>
<td>IgG*** Rarely low avidity can persist for a year</td>
</tr>
<tr>
<td>IgA</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>IgE</td>
<td>Absent</td>
<td>Present</td>
<td>Absent</td>
</tr>
</tbody>
</table>

**Toxoplasmosis: Fetal Ultrasound Findings**

- Calcifications
- Brain
- Liver
- Growth Restriction
- Microcephaly
- Splenomegaly
- Ventriculomegaly
- Hydrops
Toxoplasmosis: Ultrasound findings

Toxoplasmosis: Neonatal Findings
- Hepatosplenomegaly
- Chorioretinitis
- CNS injury
- Salivary
- Mental Retardation

Toxoplasmosis: Treatment
Conclusion

- Congenital Infections leading to CNS abnormalities
  - Zika, CMV, toxoplasmosis
  - Exposure or symptoms
  - Ultrasound abnormalities
  - Low amniotic

- Diagnostic
  - Maternal serology
  - Positive Culture
  - Amniocentesis