Congenital Infectious Encephalopathies

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  – NIH/NIAD Collaborative Antiviral Study Group (CMV, HSV)
Objectives

1. Discuss the term “TORCH”
2. Describe congenital infections from presentation, evaluation, diagnosis, and discuss prognosis
   1. Cytomegalovirus
   2. Lymphocytic Choriomeningitis Virus (LCMV)
   3. Parvovirus B19
   4. Toxoplasma
   5. Zika virus
3. Recognize emerging infectious threats to the mother/fetus and infant
Congenital infections

• Are likely under-recognized
• May not get treated
• May be preventable in some cases

• Can contribute to **substantial abnormalities of the developing brain**
  – **Destructive lesions** - intracranial hemorrhage, calcifications, reduced cerebral growth
  – **Malformative lesions** - schizencephaly, polymicrogyria, cortical dysplasia
2016 Top 25 Neurology Diagnoses

Total Cases by Diagnosis

Ventriculomegaly
Microcephaly/Small Head
Absent Septum
Agenesis of the Corpus
ZIKV
mega cisterna magna
choroid plexus cysts
cerebellar hypoplasia
Intracranial Cyst
Assymetric Venticles
Posterior Fossa
NTD, unspecified
Macrocephaly
Hypoplastic Vermis
Dandy-Walker
Intraventricular
Spine Abnormality
Holoprosencephaly
Hypoplastic Corpus
Arachnoid Cyst
Abnormal Brain Stem
Delayed Cerebral
Myelomeningocele
Aqueductal Stenosis
Colpocephaly

= Cases in which infectious etiologies should be considered
Perinatal Infection: “TORCH”- No longer works!

- **Toxoplasmosis**
- **Other Agents**
  - Hepatitis B
  - HIV
  - Parvovirus
  - Varicella Zoster Virus (VZV)
  - Lymphocytic Choriomeningitis Virus (LCMV)
  - Enterovirus (EV)
  - Syphilis
  - Zika virus (ZIKV)
- **Rubella**
- **Cytomegalovirus (CMV)**
- **Herpes Simplex**

- **Term “TORCH” coined in 1971**
- **Introduction of “TORCH-titers”**
- **Since then, new infectious etiologies appreciated**
- **“Other” list is very long**

*TORCH is no longer accurate since 2014!*
## Sequelea of Congenital Infections

<table>
<thead>
<tr>
<th>Sequelea</th>
<th>Toxo</th>
<th>Rubella</th>
<th>CMV</th>
<th>HSV</th>
<th>EV</th>
<th>Syphilis</th>
<th>VZV</th>
<th>HIV</th>
<th>LCMV</th>
<th>Zika</th>
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## Discriminating CNS Abnormalities

<table>
<thead>
<tr>
<th>Sign</th>
<th>Toxo</th>
<th>Rubella</th>
<th>CMV</th>
<th>HSV</th>
<th>EV</th>
<th>Syphilis</th>
<th>VZV</th>
<th>Parvo</th>
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<tr>
<td>Microcephaly</td>
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<td>Hydrocephaly</td>
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<td>Calcifications</td>
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</table>

5 diffuse  6 periventricular  7 basal ganglia  8 subcortical
### Comparison of cerebral calcifications in congenital infections

<table>
<thead>
<tr>
<th>Infection</th>
<th>Cerebral calcifications</th>
<th>Widely spread</th>
<th>Periventricular</th>
<th>Basal ganglia</th>
<th>Gray white matter junction</th>
<th>Cortical</th>
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<tbody>
<tr>
<td>ZIKV</td>
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<tr>
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<td>Rare</td>
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<tr>
<td>CMV</td>
<td>+++</td>
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</tbody>
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+ = infrequent  
++ = common finding  
+++ = Frequently reported and severe  

Levine D et al, 2017
Periventricular vs. Gray-white matter junction calcifications

 Congenital CMV

 Congenital ZIKV

Levine D et al, Radiology, 2017
Case 1: Three week old infant with failed newborn hearing screen presents to ID clinic

- CC: Evaluate for congenital CMV
- HPI:
  - Born at term with birth weight 3.14kg by SVD.
  - No complications of pregnancy or delivery.
  - Failed hearing screen in right ear by ABR testing
  - Mother requested testing for congenital CMV infection
Testing results - Infant

- **CMV PCR** in urine at 2 weeks of age - **positive** (>1,000,000 copies)
- **CBC** normal (no thrombocytopenia), **CMP** normal
- **Ophthalmology** - normal exam with no evidence of chorioretinitis.
- **Repeat ABR** performed - failure of right ear
- **Cranial US** - multiple bilateral periventricular cysts and calcifications c/w congenital CMV infection
- **Brain MRI** - subependymal cyst of the temporal horns of the lateral ventricles. Suspicious for polymicrogyria
Imaging findings of congenital CMV

Sagittal Cranial US-periventricular cysts (upper left image) and linear echogenic calcification (lower left image)

Brain MRI-subependymal temporal horn cysts, also suspicious areas of polymicrogyria (upper right image-axial T2 and lower right coronal T1).
Additional history

• Father diagnosed with CMV infection when mother was pregnant at 8 weeks gestation
  – Asymptomatic
  – Elevated transaminases noted on routine lipid screening
  – CMV IgM positive and CMV IgG negative

• Maternal testing results: (at 8 and 15 weeks gestation)
  – CMV IgM negative and CMV IgG positive
  – Mother was told she could not have CMV infection
  – *No CMV IgG avidity testing performed in mother*

  ▪ Rubella immune/RPR NR/GBS/HIV/HepB/GC/Chlamydia negative
Treatment - Infant

- Oral valganciclovir 16mg/kg bid for 6 months
  - Kimberlin and NIH Collaborative Antiviral Study Group randomized trial, NEJM 2015
- Monitor for toxicity with CBC and CMP
  - Initially weekly, then spaced to monthly if no toxicities
- Formal audiology evaluation to better evaluate hearing loss, to be repeated at least every 6 months
- Neurology clinic evaluation
- Follow up eye exam in 6 months
CMV: Transmission

- Passed from person to person through close contact with body fluids
  - Saliva
  - Semen, Vaginal fluid
  - Blood
  - Urine
  - Tears
  - Breast milk

- Transmitted by contact with infected fluids by inoculation via mucous membranes (eyes, nose or mouth)

- CMV also transmitted via sex, breastfeeding, blood transfusions and organ transplantation
CMV Epidemiology

• 50-80% of adults in the United States have acquired CMV infection (seropositive) by age 40
  – 40-60% in higher SES
  – 80% in lower SES

• Most people who acquire primary CMV infection (adults and children post-natal) are completely asymptomatic

• If symptomatic
  – Immunocompetent:
    • Mononucleosis syndrome, flu-like illness
    • Retinitis rare
  – Immunocompromised - severe
    • Bone marrow suppression, colitis (HIV), retinitis
CMV Exposure for Seronegative Pregnant Women

- Daycare centers are a significant source of CMV infection.
  - Up to 70% excretion rates in children 1-3 years of age
  - Children <3 years of age with postnatally acquired CMV infection excrete CMV in their urine/saliva for 6 - 42 months!
- Seronegative mothers with children in group daycare are at significant risk of acquiring CMV infection
  - At least 50% seroconvert within 1 year of their child’s CMV infection
- Preventative measures:
  - Hand Hygiene after diaper changes
  - Pregnant personnel should use universal precautions
Congenital CMV: Epidemiology

• Most common intrauterine/congenital infection
  – 1% of all live births affected
  – 9,000 infants per year in US
  – Economic burden > $2 billion annually US

• Vertical transmission to fetus at any stage of gestation
  – Highest risk in first ½ of gestation
  – Primary maternal infection:
    • 50% transmission to fetus
  – Reactivation of infection:
    • 1% transmission to fetus
    • Pre-existing maternal immunity confers significant, but not full protection against congenital infection or disease
Congenital CMV: Clinical Significance

• **Symptomatic at birth (10% of infected infants)**
  – 40-60% with severe neurologic sequelae
    • Sensorineural deafness
    • Seizures
    • Mental retardation
    • Chorioretinitis, optic neuritis, microphthalmia
    • Microcephaly, polymicrogyria
  – 10% fatal in early infancy

• **Asymptomatic at birth (90% of infected infants)**
  – 10-15% present later with neurological sequelae
    • Developmental Delay
    • Progressive sensorineuronal hearing loss

• Congenital CMV infection is responsible for 20-25% of all cases of hearing loss in young children
Congenital CMV: CNS malformation

- Most critical period for malformations and disruptions is the third to eighth week of gestation
  - Microcephaly
  - Polymicrogyria

- CMV infection in the third trimester can cause encephalitis
CNS Manifestations of Congenital CMV Infection

Hydrocephalus, periventricular calcifications, thin cortical mantle, periventricular calcifications
Atrophic cortex, dilated ventricles, periventricular calcifications
CMV Chorioretinitis

Cataract

“Blueberry Muffin” Rash: Extramedullary Hematopoesis
Congenital Cytomegalovirus: Lab Diagnosis

- Must be diagnosed in first 3 weeks of life

- CMV culture, shell vial, or PCR
  - Urine
  - Blood
  - CSF

- Serology - less helpful
  - CMV IgG - maternal transfer
  - CMV IgM
Screening for CMV in Pregnant Women

- Still controversial in US
- Neither ACOG or CDC recommend universal screening
- Why?
  - Seropositivity does not rule out risk of infection to fetus
  - Reactivation, Infection with new strain
  - Dx of *in utero* CMV infection (using amniotic fluid PCR) does not necessarily predict symptomatic disease or sequelae
  - No established evidence in randomized trials for efficacy of preventing fetal CMV infection in pregnant patients
  - Maternal screening may cause undue anxiety
  - **Not cost-effective unless treatment, such as CMV-specific hyperimmune IVIG, could result in >50% reduction of symptomatic infection**
Prenatal Serologic Dx of CMV in Mother

- **Knowing serostatus PRIOR to pregnancy is ideal**
- **If CMV IgG and IgM both negative**
  - identifies seronegative mother at risk for infection
- **If CMV IgG and IgM both positive**
  - either primary infection or reactivated disease
- **If only CMV IgG positive; IgM negative**
  - Prior infection
  - But if obtained for first time from pregnant mother with symptoms or abnormalities on prenatal imaging, avidity may assist
- **IgG Avidity can help sort out**:
  - Low avidity IgG = acute or recent infection
  - High avidity IgG = infection in past
  - If high avidity IgG present in 1st trimester (first 12-16 weeks gestation), low risk of CMV transmission to fetus
Serologic Diagnosis During Pregnancy

- Anti-CMV IgG
  - Primary infection
  - Secondary infection

- Anti-CMV IgM
  - First CMV- M and -G positive bleeds
  - CMV-M positive (secondary infection)

Amount of IgG/IgM Antibody or Avidity vs. Time
Amniotic Fluid CMV PCR

• Sensitivity 90-98%; Specificity 92-98%
• Must be done >6 weeks after symptom onset and >22 weeks gestation
• Threshold AF CMV viral load predictive of symptomatic disease is not known; conflicting results:
    • $\geq 10^3$ copies/mL; 100% risk for congenital infection
    • $\geq 10^5$ copies/mL; predictive of symptomatic congenital infection
    • <500 copies/mL; unlikely symptomatic congenital infection
  – Other groups
    • No clear cutoffs; considerable overlap
    • If negative, infection unlikely
Prenatal Treatment with CMV Hyperimmune Globulin: The Jury is Still Out

• 2005 Uncontrolled Prospective study: Nigro et al NEJM 2008
  – Monthly infusions to mothers with proven infection suggested infusion was safe and could prevent (adjusted odds ratio 0.32) and treat (0.02) fetal infection

• 2014 Randomized Trial: Revello et al, NEJM 2014
  – Not protective, and possible increased adverse events

• Still one randomized, controlled clinical trial involving CMV hyperimmune globulin ongoing (NCT01376778)

• Cochrane Review underscores lack of data from randomized controlled studies to assess efficacy for prevention of intrauterine transmission and adverse outcomes
The Future: Active Congenital CMV Research - 1

• ClinicalTrials.gov:
  – 34 trials (US, China, Israel, Italy) focusing on Congenital CMV
    • 9 completed; 12 active/10 recruiting

Targeting Prenatal Period:
  – Identification of Prenatal/fetal biomarkers
    – Maternal viral load, cytokines, genetics
  – Prenatal Intervention/Treatment
    • Prevention with Behavioral modification (hygiene) to avoid infection for seronegative moms
    • Prevention with vaccine
    • Prevention with passive immunity
      – Cytogam - Multicenter RCT
      – Standard IVIG
    • Prevention using antiviral therapy: Oral Valacyclovir - RCT
Targeting Postnatal Period:

- Postnatal Universal Screening and Treatment
  - Screen (PCR) all newborns: Saliva or Urine PCR
  - Screen (PCR) subset of newborns who fail newborn hearing screen

- Postnatal Treatment
  - Monoclonals
  - Newer antiviral agents
  - Expansion of treatment to asymptomatic infants - valganciclovir
  - Expansion of treatment to beyond the neonatal period
    - Screen and treat subset of infants who develop later sensorineuronal hearing loss (NIH/CASG 204)
      - All infants 1 month-4 years of age with sensorineuronal hearing loss eligible
      - Neonatal blood spot PCR for retroactive dx of cong CMV
      - Randomize to treatment with valganciclovir for 6 weeks vs. placebo
Case 2: Four month old male twin with seizures

- 37 week twin Gestation, Homeless mother
- Prenatal Eval:
  - US at 29 weeks: Ventriculomegaly noted
  - Fetal MRI:
    - Extensive loss of cortex and white matter, multicystic encephalomalacia within anterior, middle and posterior cerebral distribution
    - Lateral ventricles moderately enlarged – ex vacuo
- Newborn exam unremarkable: Wt 5%-ile; HC: 5%-ile
- Twin sister, normal
- Placenta pathology: accelerated villous maturation and chronic villitis of unknown etiology
- Testing at Birth:
  - CMV PCR urine negative
  - Toxoplasma IgG/IgM negative on mother and infant
  - HIV/RPR/HepB/GBS all negative
Fetal MRI at 29 weeks gestation
Postnatal MRI at 6 weeks of age – normal neuro exam

- Extensive cerebral volume loss in bilateral parietal lobes with large areas of cystic encephalomalacia. Frontal/parietal/occipital polymicrogyria
- Increased T1 signal along ventricular walls suggestive of mineralization
Abnormal movements at 3 months of age

- EEG with right frontal slowing, rare discharges, no seizures
- Normal hearing evaluation
- No visual tracking noted on exams → Ophthalmology consulted
  - Diffuse pigmentary changes throughout the macula
- Additional testing:
  - LCMV IgG positive 1:256
  - LCMV IgM negative
Lymphocytic Choriomeningitis Virus (LCMV)

- Single-stranded RNA arenavirus
- Common house mouse is natural host and reservoir for the virus
  - Hamsters can be reservoirs as well
- Shed in large amounts in urine, feces, saliva, and nasal secretions
- Humans acquire via inhalation of aerosolized particles → initial replication in lung tissue → viremia → tropism for neural tissues
  - Transplacental migration can occur during viremia
- Diagnosed by immunofluorescent antibody testing (serum or CSF) – ARUP Labs commercially
LCMV Clinical Manifestations and Diagnosis

- Acquired/Postnatal infection:
  - Asymptomatic/mild in as many as 1/3 of pts
  - Tropism for meninges and choroid plexus
  - Classically presents as aseptic meningitis

- Congenital Infection:
  - Predilection for neural parenchyma
  - Structural cerebral anomalies
  - Microcephaly, Intracranial Calcifications
  - Retinal damage common (80%)
  - Long term/life-long sequelae

- No treatment available
- Supportive Care

Bonthius, *Seminars in Pediatric Neurology*, 2012,
How much of a problem is LCMV?

• Incidence of congenital infection unknown
  – Fewer than 100 cases in literature
  – Not routinely considered

• Adult seroprevalence
  – LCMV 5%
  – CMV 45-100%
  – Syphilis 0.71%

• Wild mouse infection endemic in temperate regions
  – 10% in some inner cities
  – Not always a clear exposure history for congenital cases

• Horizontal human transmission has not been documented
Case 3: Cerebellar hypoplasia following fetal anemia

- 32-year-old G3P1 woman with **fetal ascites** on routine obstetric US at **20 weeks**
  - Maternal testing
    - Positive for human Parvovirus B19 IgG and IgM.
    - Negative IgM for CMV, Toxo, HSV-1, all Positive IgG serologies

- **At 21 weeks**
  - Stable ascites
  - **Anemia on Doppler US** - Intrauterine blood transfusion was performed

- **At 23 weeks**
  - **Cerebellum hypoplastic** on US - mother referred to Fetal Medicine Institute for fetal MRI

- Serial follow-up US confirmed normalization of the cerebral Doppler parameters and resolution of ascites.

Sanapo et al, J Matern Fetal Neonatal Med 2017
US and MRI imaging in case with congenital Parvovirus infection

Sanapo et al, J Matern Fetal Neonatal Med 2017
Parvovirus Infection: Epidemiology

• SS DNA Virus

• Exclusively infects humans
  – Contact with respiratory secretions, blood

• Serologic evidence of infection
  – 5-10% Young children
  – 50% Adults
  – 90% Older adults

• Childcare center/school outbreaks in Springtime
Parvovirus: Postnatal Clinical Presentation

- Suppression of RBC production
- Fever: 15-30%
- Rash - “Slapped Cheek” Disease; Erythema Infectiosum
- SS disease - Aplastic Crisis
Parvovirus: Fetal Clinical Presentation

- Risk of transplacental infection in pregnant women is 30%
- Risk of fetal death ~25% for first trimester infection
- Fetal Hydrops (especially 2nd trimester exposure)
  - 10-20% of all cases of non-immune hydrops fetalis
- CNS injury
  - Parenchymal calcifications
  - White matter injury and cerebral infarction
  - Cortical abnormalities- dysplasia, heterotopias, polymicrogyria
- Fetal anemia, thrombocytopenia, & congestive heart failure
Fetal Hydrops - Parvovirus

- Pregnant women:
  - Serology
  - Ultrasound
  - if exposure occurs and seronegative
Parvovirus B19 Management and Prevention

• No specific treatment or vaccine
• Frequent hand washing to prevent infection especially in pregnant women
• Some countries recommend avoiding a workplace where outbreak is occurring
Case 4: Hydrocephalus in a full term newborn twin

- Full term Twin B Infant born to 30 yo G2P3 female
- Hydrocephalus noted 3 weeks prior to delivery
- Neurosurgery immediately placed VP shunt
  - LP 250 WBC, 145 RBC, Protein 1750, Glu <1
- Maternal History:
  - Pregnancy uneventful, all maternal prenatal labs unremarkable
  - No flu-like symptoms during pregnancy
  - No raw meat ingestion
  - Has cat
- Infant Exam:
  - Lethargic ill-appearing
  - Macrocephalic
  - No icterus, jaundice, organomegaly
  - No rash
- Ophtho exam: Chorioretinitis
Hydrocephalus in congenital Toxo infection
Toxoplasmosis: Epidemiology

- Ubiquitous
- Intracellular parasite: *Toxoplasma gondii*
- Incidence in US: 1: 1000-10,000 live births
  - Risk highest early in pregnancy
- Reservoir: House cat and other animals
- Source of infection:
  - Cat feces: accidental ingestion of oocysts by handling litterbox, gardening, eating unwashed fruits/vegetables
  - Consumption of raw or undercooked meats
  - Uncommon: water, blood, transplantation
Toxoplasmosis: Epidemiology and Transmission
Congenital Toxoplasmosis: Clinical Manifestations

- Asymptomatic at birth: 70-90%
- Mental retardation/Developmental Delay
- Microcephaly/Hydrocephalus
- Cerebral Calcifications (Diffuse)
- Chorioretinitis/Visual Loss
- Hearing Loss
Congenital Toxoplasmosis: Diagnosis and Treatment

**Diagnosis:**
- Serology: Toxoplasma Serology Lab, Palo Alto, CA
  - Maternal/Infant Panel:
    - Maternal: IgG, IgM, Avidity or AC/HS
    - Infant Panel: IgG, IgM, IgA, IgE, AC/HS
  - Amniocentesis:
    - PCR > 18 weeks (Sensitivity 97%, Specificity 100%)

**Treatment:**
- Prenatal:
  - Spiramycin reduces transmission from mother to fetus
    - 23% transmission in treated vs. 60% in untreated
- Postnatal:
  - Pyrimethamine, sulfadiazine, with folinic acid for 1 year
  - Close monitoring (weekly) for toxicities
Toxoplasmosis- Control Measures

• Pregnant women
  – Avoid exposure to cat feces
  – Gloves when changing litter (if contact is unavoidable)

• Daily changing of cat litter
  – Oocysts are not infective during 1-2 days after passage

• Eat well cooked meats
  – Pork, lamb, venison

• Wash fruits & vegetables

• Wash hands after gardening or contact with soil
Case 5: Fever and Rash Illness in Pregnancy

- 33 yo G1P0 U.S. resident vacationed for one week in Belize, Guatemala and Cancun at 11 weeks gestation
- 2 days after return to the US, she developed illness lasting 5 days:
  - Low grade fever
  - Erythematous maculopapular rash
  - Myalgia
  - Mild photophobia
  - No joint symptoms

-Husband (also on trip) developed identical symptoms within same time frame
Case presentation (continued)

Obstetrical Evaluation:
- 13 weeks gestation (1 week post-symptoms)
  - Fetal ultrasound normal

- 16 and 17 weeks gestation (4 and 5 weeks post-symptoms)
  - Repeat fetal ultrasounds normal
  - Serum Zika IgM and IgG positive, Zika RT-PCR positive

- 19 weeks gestation (7 weeks post-symptoms)
  - Repeat fetal ultrasound abnormal
  - Referred to CNHS Fetal Medicine Institute for multidisciplinary evaluation
  - Fetal MRI: Multiple CNS abnormalities detected
  - Amniotic Fluid Zika RT-PCR positive
Fetal MRI at 20 weeks gestation: Severe atrophy of cortical mantle

- Normal lamination pattern absent
- Subplate zone largely undetectable

Fetal MRI at 20 weeks: Small Corpus Callosum

- No focal destructive lesions in cortex or white matter
- Cerebellum normal in size and appearance
Background- ZIKV

- Zika virus (ZIKV) quickly emerged as a cause of fetal/infant microcephaly in late 2015
- Flavivirus transmitted by mosquitos and sexually–related to Dengue, West Nile Virus
- Zika remains a threat in Central/South America

Messina JP et al., eLife 2016;5:e15272
The Risk of Fetal Brain Damage Depends on Timing of Infection

- Prospective study of **555 fetuses/546 pregnancies**
  - French Territories of the Americas
  - Symptomatic cases with + PCR test

- Findings:
  - 28 (5%) not carried to term or stillborn
  - 39 (7%) fetuses/infants with **neurologic ± ocular**
    abnormalities possibly related to ZIKV
  - **Neurologic and/or ocular abnormalities** were more common in first trimester (12.7%) infection vs. second (3.6%), or third trimesters (5.3%) ($P = .0001$)

Hoen et al., NEJM 2018

- Apoptosis of intermediately differentiated post-migratory neurons in neocortex
- Mature neurons are less vulnerable to ZIKV infection than immature cells

Nestin/Sox 2 = Neural Progenitor Cells  
*Tbr2  =  Intermediate Progenitor Cells  
*DCX  =  Immature Neurons  
NeuN  =  Mature Neurons
5 Features of Congenital Zika syndrome rarely seen in other congenital infections

1. Severe microcephaly with partially collapsed skull configuration
2. Thin cerebral cortical mantle with subcortical calcifications
3. Focal pigmentary retinal mottling with macular scarring
4. Congenital contractures (arthrogryposis)
5. Significant early hypertonia and symptoms of extrapyramidal involvement

Moore CA et al, JAMA Pediatri 2017
Abnormal skull shape in ZIKV

Postnatal Head CT
Levine D et al, Radiology 2017

Postnatal Head CT with multi-planar reformations
De Souza A, et al, Childs Nerv Sys, 2017
Cerebral Calcifications in ZIKV

Postnatal head CT (Images a-c)

Postnatal brain MRI (Image e)

Fetal US (Image d)

de Souza A, et al, 2017
Congenital ZIKV arthrogryposis

De Souza A, et al, 2017
Infants with ZIKV microcephaly
- 33% have eye findings (focal pigmentary mottling, chorioretinal atrophy, optic nerve abnormalities, iris coloboma, lens subluxation)
- 6% have sensorineural hearing loss
- ≤50% have seizures

Acute seizures can be seen in the setting of cerebral infarction/vasculopathy associated with ZIKV

Other: encephalitis/meningoencephalitis, ADEM, myelitis, sensory polyneuropathy and sensory neuronopathy

### Zika Evaluation and Testing - Newborn/Infant

<table>
<thead>
<tr>
<th>Zika PCR</th>
<th>Zika IgM</th>
<th>Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>Any result</td>
<td>Confirms congenital Zika virus infection</td>
</tr>
<tr>
<td>Negative</td>
<td>Non-negative</td>
<td>**</td>
</tr>
<tr>
<td>Negative</td>
<td>Negative</td>
<td>**</td>
</tr>
</tbody>
</table>

** Interpretation should be in the context of infection during pregnancy, maternal test results, clinical findings, and any confirmatory testing with PRNT

https://www.cdc.gov
The “Zika exposed” newborn - 3 categories of concern

CZS = congenital Zika syndrome

- Newborn with CZS birth defects and mothers with possible Zika exposure (regardless of test results)
- Newborn without CZS birth defect and mother with lab evidence of Zika in pregnancy
- Newborn without CZS birth defect born to mother without lab evidence of Zika in pregnancy
Evaluation of a newborn with congenital Zika syndrome birth defects or newborn without CZS born to mother with positive or indeterminate testing

- Zika virus testing
- Standard newborn comprehensive exam
- Cranial US within 1 month of age
- Comprehensive ophthalmology exam within 1 month of age
- Auditory brainstem response (ABR) test by 1 month of age if infant passed newborn hearing screening only by the otoacoustic method

Adebanjo et al., MMWR, Oct 20, 2017; https://www.cdc.gov
Obtain multi-specialty consultations when available

- Infectious disease
- Clinical geneticist
- Neurologist
  - Consider **Brain MRI** within 1 month of age
  - EEG if concern for seizures
- Referral to a developmental specialist/early intervention services
- Other referrals based on clinical findings
- Ensure available family support

Adebanjo et al., MMWR, Oct 20, 2017; https://www.cdc.gov
Children’s National Congenital Zika Virus Program

Since 2016:

- Clinical consultations on 90 mother/infant pairs
- Enrollment of additional 85 mother/infant pairs for research study
- Zika-exposed and infected pregnant women/fetuses and infants
Findings of Congenital Zika Syndrome in fetus at 25 weeks

• Fetal MRI and US
  – Severe microcephaly
  – Thin cortical mantle
  – Ventriculomegaly
  – Arthrogryposis

Children’s National, unpublished
Fetal and Postnatal Brain Imaging for the Detection of ZIKV Encephalopathy in the Fetus/Newborn

- Children’s National study in collaboration with Biomelab in Barranquilla, Colombia

- Objective: To evaluate concomitant brain MRI and US abnormalities in fetuses/newborns with in utero ZIKV exposure/infection
Methods

• **Prospective study of pregnant women**
  – Two sites: Washington, DC (*travel-related*); Barranquilla, Colombia (*endemic*)
  – Clinical diagnosis with or without lab confirmation (ZIKA PCR, IgM and/or IgG)
  – Enrollment 7/13/16 to 06/28/17

• **Longitudinal concomitant neuroimaging**
  – Fetal US and MRI performed in 2\textsuperscript{nd}-3\textsuperscript{rd} trimesters
  – Standardized imaging protocol
  – Central interpretation (Children’s National)

• **Postnatal imaging**
  – Unsedated newborn brain MRI and cranial US
Neuroimaging Study Results

• 82 pregnant women-fetal dyads
  – 79 in Barranquilla, Colombia
  – 81 with symptomatic ZIKV infection at 8.4 ± 5.7 weeks GA
  – Subsequent lab testing confirmed evidence of Zika infection
    • All had positive IgG (standard methodology); one had positive PCR
    • Many had positive IgM by standard and/or NS1 methodology

• Fetal Imaging
  – First fetal MRI and US at 25.1 ± 6.3 weeks GA
  – Second fetal MRI and US at 31.1 ± 4.2 weeks GA (n=36)

• 80 cases resulted in live term birth
  – 1 case was terminated at 23.9 weeks GA (fetal heterotopias)
  – 1 case resulted in near-term fetal demise
  – 1 live born infant died at 3 days of age

Mulkey et al, In Press
Fetal-Neonatal Brain MRI and US Results

Summary

• 3 of 82 (4%) cases had abnormal fetal brain findings
  – All abnormal fetal cases were severe
• 1 case had an isolated postnatal brain MRI abnormality (likely ZIKV related)
• 37% of 57 cases (that had postnatal cranial US) had cranial US findings of choroid plexus cysts, subependymal cysts, and/or lenticulostriate vasculopathy

Mulkey et al, *In Press*
### Research Case 1

<table>
<thead>
<tr>
<th>GA (wks)</th>
<th>Fetal MRI</th>
<th>Fetal MRI Images</th>
<th>Fetal US</th>
<th>Fetal US Images</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 6/7</td>
<td>Multifocal irregularity of the bilateral occipital germinal matrix/heterotopias (blue square)</td>
<td><img src="image1" alt="Fetal MRI Images" /></td>
<td>Normal</td>
<td><img src="image2" alt="Fetal US Images" /></td>
</tr>
<tr>
<td>22 3/7</td>
<td>Heterotopias (blue square), unexpected cortical anomaly in the left parietal cortex (red circle)</td>
<td><img src="image3" alt="Fetal MRI Images" /></td>
<td>Normal, slight decrease in HC</td>
<td><img src="image4" alt="Fetal US Images" /></td>
</tr>
</tbody>
</table>

*HC = head circumference*

*Mulkey et al, In Press*
<table>
<thead>
<tr>
<th>GA (wks)</th>
<th>Fetal MRI</th>
<th>Fetal MRI Images</th>
<th>Fetal US</th>
<th>Fetal US Images</th>
</tr>
</thead>
<tbody>
<tr>
<td>28 5/7</td>
<td>Chiari II malformation and parietal encephalocele (red arrow)</td>
<td><img src="image1" alt="Fetal MRI Image" /></td>
<td><img src="image2" alt="Fetal US Image" /></td>
<td><img src="image3" alt="Fetal US Image" /></td>
</tr>
<tr>
<td>37</td>
<td>Microcephaly, Chiari II malformation and parietal encephalocele</td>
<td><img src="image4" alt="Fetal MRI Image" /></td>
<td><img src="image5" alt="Fetal US Image" /></td>
<td><img src="image6" alt="Fetal US Image" /></td>
</tr>
</tbody>
</table>

**HC** = head circumference

Microcephaly (HC < 3rd %), Chiari II, encephalocele

Mulkey et al, *In Press*
## Research Case 3

<table>
<thead>
<tr>
<th>GA (wks)</th>
<th>Fetal MRI</th>
<th>Fetal MRI Images</th>
<th>Fetal US</th>
<th>Fetal US Images</th>
</tr>
</thead>
<tbody>
<tr>
<td>32</td>
<td>Abnormal midbrain tectum, cerebellar dysplasia, lateral VM, thin white matter and corpus callosum, heterotopias, cortical dysplasia</td>
<td><img src="image1.jpg" alt="Fetal MRI Images" /></td>
<td>Mild VM (11.7mm), small cerebellum, HC 38th percentile</td>
<td><img src="image2.jpg" alt="Fetal US Images" /></td>
</tr>
<tr>
<td>36</td>
<td>Similar appearance of multiple brain changes</td>
<td><img src="image3.jpg" alt="Fetal MRI Images" /></td>
<td>Severe VM (17mm), small cerebellum, HC 3.6th %</td>
<td><img src="image4.jpg" alt="Fetal US Images" /></td>
</tr>
</tbody>
</table>

VM = cerebral lateral ventriculomegaly; HC = head circumference

Mulkey et al, *In Press*
# Research Case 4

<table>
<thead>
<tr>
<th>GA (wks)</th>
<th>MRI Result</th>
<th>Fetal-Neonatal MRI Images</th>
<th>US Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>18 5/7</td>
<td>Normal</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>22 5/7</td>
<td>Normal</td>
<td></td>
<td>Normal</td>
</tr>
<tr>
<td>28 3/7</td>
<td>Normal</td>
<td></td>
<td>Normal</td>
</tr>
</tbody>
</table>

**Post-natal (16 days)**

Area of chronic infarction (at least 4-6 weeks prior by imaging) in the right parietal region (red arrows)

VM = cerebral lateral ventriculomegaly; HC = head circumference

Mulkey et al, Ped Neurol, 2018
Postnatal Cranial US Findings

• 21 of 57 (37%) infants that had postnatal cranial US had a finding

• Germinolytic or choroid plexus cysts on postnatal head US (16 infants)

• Lenticulostriate vasculopathy (4 infants)

Mulkey et al, In Press
Neurodevelopmental Outcome Research-Six-month Infant Evaluations in Sabanalarga, Colombia

Photos: Drs. Cure and Mulkey explain the study to the mothers at the 6-month visit (left). Dr. Mulkey performs the AIMS exam on an infant and teaches the AIMS exam to a Biomelab research coordinator (right).

Photos by M. Arroyave-Wessel, MPH.

Mulkey et al, PAS 2018 & Child Neurology Society 2018
Interim Outcome Results at 6 months in Zika Cohort

- Normocephalic infants with normal MRI exposed to ZIKV in utero have normal neuromotor development at 6 months of age
- Lower self-care domain scores on WIDEA may result from cultural differences and not developmental delays
- 12 month follow-up is in progress

Mulkey et al, PAS 2018 & Child Neurology Society 2018
Ongoing ZIKV Surveillance

- CDC and Health Departments working to ensure multidisciplinary care of exposed infants
- Develop assessment tools
- Determine case numbers
- Develop case definitions and define full spectrum of abnormalities
- Determine long-term health needs
US Virgin Island Health Brigade March 17-25, 2018

- Provided **multi-specialty care** to infants exposed to ZIKV during pregnancy
- Hurricane damage still a major factor
- Support and education for local providers

Child neurologists (Mulkey, Bale, De Cruz, Payne)  
Ophthalmologists & Audiologists
Congenital Zika Program at Children’s National
Infant Follow-up at 18 months

• Neurologic and developmental assessments
  – Complete neurologic exam
  – ASQ and ASQ-SE
  – WIDEA

• ZIKV testing in mother and infant using NS1 methodology at 18 months
  – Determine true presence of congenital infection

• Audiology or ophthalmology follow-up when needed

• Developmental or therapy referrals as indicated
Thank you

- Congenital Zika Program and Research Support
  - Institutional funding from Children’s National Health System
  - Ikaria Fund
  - Neurologic follow-up study Thrasher Research Fund (Mulkey; Mentors: du Plessis and DeBiasi)