NEUROLOGIC INFECTIONS

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I. ACUTE FLACCID MYELITIS (AND EV-D68)

Chronology
August 2014 1st cases admitted to Children's Hospital CO (CHCO). September 2014 CDC notified after initial 9 cases and issues National Health Advisory

CDC Case Definition (current)
Patient presenting (after Aug. 2014) with: Acute limb weakness AND MRI showing a spinal cord lesion largely restricted to gray matter and spanning one or more spinal segments. Probable case: acute limb weakness AND CSF Pleocytosis ($\geq$5 wbc/mm^3)

CDC Confirmed U.S. Case Counts:
2014: 120 Cases in 34 States
2015: 21 Cases in 16 States
2016: 132 Cases in 37 States

Demographic Features

	CO Series	CDC Series)
SEX:	75% M	71% M
AGE (med):	11.5 yrs	7 yrs
	6.75-15 yrs IQR	0.4-21 yrs range
VACCINATION	92% current	
Prodrome		
Pre-CNS	3-12d (IQR 5.8-8d)	5.6$\pm$3.2 d
Fever	100%	64%
URI	92%	81%
Meningitic	83%	
Headache	58%+	

Pattern of Weakness
PROXIMAL>DISTAL
ARMS > LEGS
HYPO- or AREFLEXIC
NO SENSORY LOSS
NO SZ's/ENCEPHALOPATHY

Laboratory Findings
CSF Pleocytosis in 100% done at < 7 days, CDC 72% overall
Median 55 cells (IQR 14-62), CDC: 91 $\pm$104 cells
Elevated protein 50% (max 92 mg/dl), CDC 58$\pm$51
Normal glucose
MRI Abnormal in 100% per CDC definition
T2/FLAIR hyperintense, SC grey matter/anterior horns, longitudinal extensive and confluent (4-20 seg, median 17), occ. Increased signal ventral roots. Lesions occ in brainstem, cerebellum

EMG c/w LMN involvement: Decr CMAPs, nl SNAPs, no demyelination, denervation seen later

Virology
2014 cases temporally and geographically associated with major U.S. epidemic of EV-D68
~50% of cases had +RT-PCR EV-D68 in Nasopharynx Swabs: Clade B1
CSF RT-PCR negative, BLOOD RT-PCR negative, STOOL+ RT-PCR negative (1 positive case…)
No other virus 'signal' of EV-D68 magnitude, negative metagenomic sequencing etc.

Animal (Mouse) Model
EV-D68 2014 strains (not Fermon, Rhyne prototypes from 1962) cause paralytic disease in mice after intramuscular and intracerebral inoculation in neonatal mice; less commonly after IP or intra-nasal. Virus infects and kills anterior horn cells.

Viral antigen, genome, particles, infectious virus are found in spinal cords of mice.

Comment: Data strongly supports an association between EV-D68 and AFM and mouse model establishes strong biological plausibility. Some cases of AFM are certainly due to other viruses but EV-D68 is likely responsible form many of the AFM cases in 2014/2016 in U.S.

Therapy
Cell Culture: Fluoxetine, 3C protease inhibitors (rupintrivir (AG7088), SG85) reduce replication. No clear effect pleconaril, vapendavir (BTA798), pocapavir (V-073, SCH-48973).

Mice: IVIG and EV-D68 immune sera provide protection and therapeutic benefit. No effect of steroids (? Worse illness), fluoxetine (a viral 2C protease inhibitor). Studies of viral 3C protease inhibitors in progress (rupintrivir).

Humans: Usually receive combinational therapy- no efficacy clear for IVIG, plasmapheresis, steroids, fluoxetine in non-controlled reports. No clear efficacy with pleconaril, vapendavir, pocapavir.

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II. ZIKA VIRUS & NEUROLOGICAL DISEASE

Virology
ss RNA virus, Family *Flaviviridae*, Flavivirus genus: Includes WNV, SLEV, JEV, Dengue, YFV
Arthropod-borne virus ("Arbovirus"): *Aedes sp*. Primary vector (*albopictus, aegypti*)
Sylvatic cycle between non-human primates-mosquitos-non-human primates
Human viremia high enough to allow human-mosquito-human transmission
Brazil: reports of infection in marmosets, capuchin monkeys (2016)

History
Isolated in 1947 from a Rhesus money as part of YFV surveillance studies in Zikka Forest near Entebbe, Uganda

1947-2007: <15 reported human cases

2007: Micronesia Outbreak on Yap island
2013: French Polynesia Outbreak (>50,000 cases)
May/2015: 1st Brazilian Case Reported by Pan American Health Organization (PAHO)
Dec/2015: Puerto Rico reports endogenous transmission (~36,000 cases by Feb/2017)
Feb/2016: World Health Organization (WHO:) Public Health Emergency of International Concern (likely >1 million cases by Feb/2017)
Sept/2016: FL Dept. Hlth. Reports local transmission in Miami-Dade & Broward Counties
(214 endogenous cases by Feb/2017)
Nov/2016: TX Health Dept. reports cases in Brownsville, TX (6 endogenous by Feb/2017)
Sept/2016: Congress approves $1.1B spending bill (Requested by Pres. Obama in Feb/2016)
Nov/2016: WHO announces end of Zika "epidemic" (cases continue…)

US Case Counts (Feb/2017, CDC data)
Continental U.S.: 5040 total: 4748 travelers, 220 FL + 6 TX endogenous
318, blood donors, 44 sexually transmitted, 26 congenital, 1 lab acquired (not mine!)
FL 1069, NY 1020, CA 420, TX 306, NJ 176, PA 173, MD 130, MA 120
U.S. Territories: Puerto Rico 35,930; U.S. Virgin Islands 973, American Samoa 120

World: 53 Countries with new ZIKAV infection since 2015 (S. & C. America, Caribbean, etc).
48 Countries with endogenous transmission, 23 with congenital Zika associated syndrome cases

Transmission
Non-arthropod Alternate modes of sexual transmission identified:
April/2016: M to M sexual transmission (MMWR)
June/2016: M to F oral sex transmission (NEJM)
July/2016: F to M sexual transmission (MMWR)
Sept/2016: Asymptomatic M to F sexual transmission (MMWR)

Symptomatology:
Overall: ~20% symptomatic/80% asymptomatic
Rash 90%
Fever 65%
Arthralgia/Arthritis 65% (less severe than CHIK)
*Conjunctivitis 55% (>>Dengue or CHIK)
Myalgia 50%
Headache 45%

Overlap with Chikungunya and Dengue

Neurological Syndromes

Case reports of microcephaly followed by increased incidence especially in Northeast Brazil in some cases reported to exceed 50/1000 live births

Brazil: >10,000 “confirmed” ZIKV cases in pregnant women 2015/16
>2000 confirmed cases of microcephaly
Brasil P et al (NEJM/2016): Study of 88 women symptomatically infected wks 5-38 pregnancy
29% had Doppler Ultrasound evidence of fetal abnormalities
premature fetal death (36, 38 wks GA), microcephaly, ventricular calcifications, ventriculomegaly, encephalomalacia, developmental abnormalities (pachygyria/hypogyration, lissencephaly, corpus callosum dysgenesis)

WHO: 23 countries have reported “congenital syndrome associated with ZIKAV infection” (Its not just microcephaly…)

Laboratory: Virus infects cortical neural progenitor cells and causes apoptosis. Brain organoid and related models show infection results in loss cells, inhibits replication, reduces growth & ‘development’

Ocular abnormalities:
Chorioretinal atrophy, pigment mottling, optic nerve atrophy. Blindness and decreased visual acuity

Guillain-Barre Syndrome
1st reported after review of Polynesia cases (Cao-Lormeau, Lancet 2016)
88% Preceding viral syndrome c/w ZIKV, ~6d later GBS
weakness (74%), hyporeflexia (62%), bilat facial palsy 33% initial-60% @nadir
62% dev. Walk difficulty, 33% resp. impairment
31% had anti-ganglioside, 48% at f/u
?Acute Motor Axonal Neuropathy (AMAN) pattern by electrophysiology

Colombian cases (Parra, NEJM 2016)
Presentation: Limb weakness (97%), Ascending paralysis (82%), hypo-reflexia (94%), paresthesias (76%), facial palsy (presentation 32%- later 50%)
Electrophysiology: 78% AIDP (only 2% c/w AMAN).
Albumino-cytological dissociation(82%)

Respond to IVIG with pattern similar to non-ZIKV GBS

Diagnosis

RT-PCR is specific: individual TaqMan RT-PCR or Trioplex RT-PCR assay for ZIKV, Dengue, Chik tests all 3 viruses simultaneously
Test serum AND urine in pts presenting < 14 days after illness onset- RT-PCR negative after acute phase except possibly in pregnant women with infected fetus?

Test CSF in neurological disease (RT-PCR, Serology)

ZIKA MAC-ELISA tests for IgM: Note flavivirus cross-reactions
Use in suspected cases when RT-PCR negative (a +RT-PCR is diagnostic)
Typically IgM+ by d4 p-onset and persists for ~ 3 months
Note issue of heterologous cross-reaction with other flaviviruses (but not CHIK- an alphavirus). Positive tests may require specialized testing for plaque-reducing neutralizing (PRNT) Abs
+CSF IgM is c/w intrathecal synthesis and ZIKV CNS disease

GBS: ~40% RT-PCR+ any fluid (urine>>>CSF>serum); of those tested 67% urine +, 10% CSF+, 3% serum+
Serology: IgG+ or IgM+ in any fluid: 86%
32% IgM+ serum/19% +CSF; 93% IgG+ serum/82% +CSF

Sperm/seminal fluid may be positive in infected males

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III. HUMAN ENDOGENOUS K-RETROVIRUSES IN MOTOR NEURON DUSEASE

Human endogenous retroviruses ~ 8% human DNA

Likely remnants of integrated proviral genomes resulting from ancestral infections and accumulated in the germ line over human evolution

Prior studies suggested ALS pts express reverse transcriptase (RT) activity (a retroviral 'signature') in blood and brain tissues

Study of 11 ALS vs. 16 healthy controls found increased expression all HERV-K genes (*gag, pol, env*) in ALS postmortem brain tissue versus controls-likely entire genome expressed

env protein expression is in ALS pt. pyramidal cortical neurons and anterior horn neurons and NOT in lateral or posterior horns of SC and not in glia or white matter. Not in controls or AD brain tissue.

Expression of HERV-K genome (or just *env*) is toxic to cultured human neurons and electroporation of *env* gene in utero into mouse brain or transgenic mice expressing *env* in neurons causes neuronal changes.

HERV-K env Tg mice have loss of upper and lower motor neurons and develop motor dysfunction

Is there a distinct phenotype for HERV-K Associated MND?
How common is HERV-K in ALS?

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