Once Bitten: Multiplex Detection of Dengue, Chikungunya and Emerging Arboviruses

Jesse Waggoner, MD
Division of Infectious Diseases and Geographic Medicine
Stanford University School of Medicine
Disclosures and Conflicts of Interest

• None

Biases

• Our laboratory has developed molecular diagnostics for dengue, chikungunya, and multiplex other arboviruses

• I collaborate with a number of companies in the Bay Area on dengue diagnostics
Overview

• Emerging Infectious Diseases
• Dengue Virus Re-Emergence
• Chikungunya Virus in the Western Hemisphere
• Emerging Arboviral Pathogens
• Conclusions
Emerging Infectious Diseases

• Institute of Medicine Definition (adopted by the CDC)
  • An infectious disease whose incidence in the human population has increased in the preceding two decades
  • Or one whose incidence threatens to increase in the near future.

• Viral diseases account for a large proportion of such infections

• Emerging viruses are typically divided into two groups
  • newly identified viruses
  • previously recognized viruses with an apparent increase in disease incidence
Examples of Emerging Viruses

• Alphaviruses
  • Chikungunya

• Arenaviruses
  • Lassa, Junin, Machupo, Guanarito, Chapare, Lujo

• Bunyaviruses
  • Hantaviruses, Rift Valley fever virus, Crimean Congo Hemorrhagic fever, Severe Fever with Thrombocytopenia Syndrome, Heartland

• Filoviruses
  • Ebola & Marburg

• Flaviviruses
  • Dengue, West Nile

• Picornaviruses
  • EV 68

• Paramyxoviruses
  • Nipah & Hendra
Reasons for Viral Emergence

• Adaptation to a new vector population
• Mutations that improve fitness for a new (or old) host
• Access to a naïve patient population
  • Movement of infected individuals, animals, or vectors
  • Encroachment of host populations into endemic regions
  • Introduction of unexposed hosts in known endemic areas
• Apparent emergence resulting from improved detection
Overview

• Emerging Infectious Diseases

• Dengue Virus Re-Emergence
  • Background & Virology
  • Clinical manifestations
  • Diagnostics
  • Management and Prevention

• Chikungunya Virus in the Western Hemisphere

• Emerging Arboviral Pathogens

• Conclusions
Dengue has been with us for a long time

- “Break-bone fever” appears in Spanish in 1771
  

- Benjamin Rush provides one early account following the outbreak of a febrile illness in Philadelphia in 1780

- After World War II, the first descriptions of dengue hemorrhagic fever (DHF) are reported
Dengue Virus (DENV)

- Member of the *Flaviviridae* family
- Single-stranded, positive-sense RNA genome
- Four serotypes (DENV 1-4)
  - Primary Infection: a patient’s first infection
  - Secondary Infection
    - Infection with a second, DENV serotype
    - Confers a higher risk for severe disease
DENV Transmission

- Transmitted by *Aedes spp.* mosquitoes
  - *A. aegypti*
  - *A. albopictus* – the “Asian Tiger Mosquito”

- Adapted to urban/peri-urban environments

- Day biting mosquitoes
DENV is on the Rise

- 3.6 billion people reside in endemic regions
- 390 million new dengue infections per year
- 2 million cases of severe disease
- 21,000 deaths

Nature 2013; 496(7446):504-7
World Health Organization, 2009
Global Distribution of DENV

The contour lines of the January and July isotherms indicate areas at risk, defined by the geographical limits of the northern and southern hemispheres for year-round survival of Aedes aegypti, the principal mosquito vector of dengue viruses.

World Health Organization, 2012
Distribution of *Aedes* Mosquitoes in the United States

*A. aegypti*  
*A. albopictus*
DENV Clinical Manifestations

• Asymptomatic or Minimally Symptomatic Infection

• Dengue Fever: non-specific
  • Fever/chills, headache, retro-orbital pain, myalgias, arthralgias, rash
  • Respiratory complaints
  • Nausea/vomiting, abdominal pain

• Severe Dengue
  • Hemorrhage, shock, organ dysfunction
Broad Differential Diagnosis

Many other infectious diseases may be clinically indistinguishable from dengue

- Malaria
- Leptospirosis
- Rickettsial disease
- Typhoid & Enteric Fever
- Influenza
- Chikungunya
- O’nyong-nyong
- Sindbis
- Mayaro
- Yellow fever virus
- West Nile fever
- Zika
- Measles
Dengue Diagnosis

• Clinical criteria are unreliable
  • WHO, 1997: sensitivity 95.4%; specificity 36.0%
  • WHO, 2009: Sensitivity 79.9%; specificity 57.0%

• Likelihood of characteristic findings on routine laboratory tests changes over time

• A confirmed diagnosis depends on the use of accurate laboratory tests
Importance of an Accurate Dengue Diagnosis

• Laboratory confirmation of dengue has been associated with decreased mortality

• Timely supportive care reduces morbidity and mortality

• Rule-in dengue: appropriate patient triage and possibly discontinue antibiotics or antimalarials

• Rule-out dengue: consider empiric therapy and search for other likely infections
DENV Diagnostics

- **NS1 (serum, ELISA, or rapid test)**
- **Viremia (serum, RT-PCR assay)**
- **IgG (primary infection)**
- **IgG (secondary infection)**
- **IgM**

*Day of Illness*

DENV Antibody Testing

- Antibody Testing is the “gold-standard” for diagnosis
  - Requires acute (≤ 5 days) and convalescent (≥ 6 days) samples.
  - Demonstrate negative to positive IgM.
  - Or ≥4-fold increase in IgG titers (at least 7 days apart).
  - Point-of-care (POC) tests are available (outside of the USA).

- DENV Antibody Pitfalls
  - IgM does not reliably develop until the 5th day of illness and can persist for 2-3 months after exposure.
  - IgM detection alone provides only a presumptive diagnosis.
  - False positives with exposure to other Flaviviruses.
  - Standard tests cannot determine viral serotype, requires PRNT (plaque reduction neutralization test).
DENV NS1 Testing

• Dengue virus Non-Structural Protein 1
  • Glycoprotein secreted from dengue virus infected cells.
  • Detected on day 1-9 of illness.
  • Good Specificity (90-100%).
  • POC assays available.

• NS1 Pitfalls
  • Widely variable sensitivity (24 – 93.4%).
  • Sensitivity 20-30% lower in secondary vs. primary infection.

Antiviral Res 2013; 98(2)
DENV Nucleic Acid Amplification Testing

• Dengue virus RNA in *serum* or *plasma*
  • Detected on day 1-5 of illness.
  • Most common: CDC’s hemi-nested RT-PCR (Lanciotti)
  • CDC DENV1-4 rRT-PCR, FDA approved 2012

• Dengue virus RNA Pitfalls
  • Wide variety of lab-developed tests: RT-PCR, LAMP, and other isothermal methods.
  • Proficiency study of 37 laboratories performing 46 different tests - 80% reported results that showed a need of improvement.

*PLoS Negl Trop Dis* 2011; 5(9): e1309
Improving DENV Molecular Testing

- Two new rRT-PCR tests
  - Single-reaction serotype-specific: DENV Multiplex
  - Pan-DENV Assay, internally-controlled

- Both assays demonstrate increased clinical sensitivity compared to
  - Hemi-nested RT-PCR
  - CDC Real-Time RT-PCR

- No amplification of related *flavivirus* RNA: Japanese encephalitis, tick-borne encephalitis, West Nile, yellow fever, Zika, hepatitis C viruses.

*J Clin Microbiol.* 2013; 51(7):2172
FDA Cleared Tests

Two available FDA approved tests for DENV

1) InBios DENV Detect™ IgM Capture ELISA

2) CDC DENV-1-4 Real-Time RT-PCR
DENV Management & Prevention

• Management
  • Many patients can be safely followed as outpatients
  • Supportive Care
    • IV fluids
    • Transfusion as needed
    • Treatment of secondary infections

• Prevention
  • Avoid mosquito bites: repellants, protective clothing
  • Removal of breeding sites and spraying
  • Vaccines – in clinical trials
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• Dengue Virus Re-Emergence

• Chikungunya Virus in the Western Hemisphere
  • Background & Emergence
  • Clinical Manifestations & Comparisons with Dengue
  • Diagnostics
  • Management & Prevention

• Emerging Arboviral Pathogens

• Conclusions
Chikungunya Virus (CHIKV)

- Genus *Alphavirus*, Family *Togaviridae*
  - Single-stranded, positive-sense RNA viruses
  - One of the fever-arthritis alphaviruses
- Transmitted by the same *Aedes* mosquito vectors as DENV
- Three genotypes
  - West African
  - East-Central-South African (ECSA)
  - Asian
CHIKV Emergence

- **Prior to 2004**
  - CHIKV maintained in enzootic cycles between primates and aedes vectors
  - Had caused a few large documented outbreaks in the 1960s and 1970s
- **2004-2005**
  - Large outbreak of the ECSA genotype develops in Kenya and Comoros
- **2006**
  - Outbreak spreads to La Réunion
  - An Indian Ocean lineage emerges at this time with the E1-A226V mutation
  - From La Réunion, outbreak spreads to Indian and across the Pacific
- **2007, 2010**
  - Documented autochthonous transmission in Italy (2007) and France (2010)
- **2013**
  - December, 2013: first documented cases of CHIKV in the Western Hemisphere (Saint Martin)
  - As of September 26: 737,084 suspected cases; 12,052 confirmed cases in the Americas
Global Distribution of CHIK
(as of September 30, 2014)

Centers for Disease Control and Prevention
CHIKV Clinical Manifestations

• Asymptomatic Infection: less common than for DENV infections (15-25%)

• “Typical” chikungunya
  • Acute onset of fever, myalgias, and joint pains
  • Rash – similar to dengue
  • GI and Respiratory complaints are less common

• Atypical presentations – mortality rate up to 10%
  • Meningitis/encephalitis
  • Pneumonia
CHIKV: Further Considerations

- Massive outbreaks in naïve populations
  - 250,000 cases in Kenya in 2004
  - 1.3 million cases in India in a single year

- Mother to Child Transmission (MTCT)
  - Incidence ~50% for mothers with intrapartum viremia
  - Neonates develop more severe disease

- Persistent arthritis/arthralgia
  - Patients report symptoms for months to over a year after infection
  - More common among women and older patients
  - May appear very similar to RA
Presentation of Chik vs Dengue

- Patients in positive for CHIKV (117), DENV (917; 55 with DHF)
- Clinical symptoms did not accurately differentiate the two etiologies.
- Only platelets < 118,000 distinguished DENV from CHIKV with an accuracy of 89%

CHIKV Diagnostics

• Antibody Testing
  • Similar to DENV, requires acute and convalescent samples for confirmation
  • Plate-based ELISAs currently available

• Pitfalls
  • IgM is not reliably detectable until day 3 or later
  • Cross-reactions can occur with O’nyong-nyong virus
  • Not commercially available for most diagnostic laboratories
CHIKV Nucleic Acid Amplification Testing

• Numerous laboratory-developed assays have been reported, using *serum* or *plasma*
  • Includes real-time RT-PCR and isothermal techniques
  • Viral RNA can be detected over the first 7 days of illness
  • Designed to be specific for CHIKV

• Pitfalls
  • No large/high-quality method comparisons available
  • No FDA cleared assays available
  • Limited knowledge regarding time course of viremia

Viral Culture

• Both DENV and CHIKV can be cultured on a number of cell lines
  • Often performed using the A. albopictus C6/36 cell line
  • CPE is variable and cell-line dependent

• Not clinically useful
  • Requires 7-14 days for growth
  • Identification needs to be confirmed by other methods
Management & Prevention

• Management
  • Supportive care for patients with severe or atypical disease
  • MTCT: no improvements seen with C-section
  • Pain control for arthritis: NSAIDS, acetaminophen

• Prevention
  • Mosquito avoidance
  • Vaccines are currently under development, though in very early stages
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• Emerging Arboviral Pathogens
  • An Exhaustive, Virus by Virus Description of Individual Emerging Viruses in Alphabetical Order by Genus

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  • Overlapping Clinical Syndromes – Challenge for Diagnosis
  • Limitations in Available Diagnostics
  • Syndrome-based Multiplex Testing

• Conclusions
Emerging Viruses: Diagnostic Challenges

• Undifferentiated Febrile Illness (UFI): a common presentation in tropical countries
  • Many emerging viral infections present in this manner
  • Acute, systemic febrile illness without localizing signs or symptoms

• Clinical suspicion
  • In the USA, this relies on obtaining a good patient history
  • In the setting of poor (or no) diagnostics, patients often receive a clinical diagnosis of dengue or malaria
  • Very difficult to rule out other etiologies
The UFI Assay

• Single reaction, internally-controlled, multiplex nucleic acid amplification test for
  • DENV
  • Leptospira: all species
  • Plasmodium: all species with a specific call-out for *P. falciparum*
Multiplex DENV & CHIKV Detection

• Single-reaction real-time RT-PCR assays have been described
  • Includes assays that purport to serotype DENV based on melt-curve analysis
  • Isothermal methods require separate reactions

• We currently use an internally-controlled assay for pan-DENV detection and CHIKV detection
  • Can be combined with assays for other pathogens
Emerging Virus Detection with Existing Diagnostics

• “Pan” PCRs
  • PCRs and RT-PCRs designed to detect a large number of viruses have been described
  • Some reportedly use primers that amplify all members of a genus or family
  • Often insensitive
  • Still rely on the detection of known pathogens

• Existing diagnostics for related pathogens
  • Relies on “cross-reactions” or a lack of specificity
  • One example: the identification of Usutu virus
    • Patient presented with encephalitis
    • WNV RT-PCR was repeatedly positive but with a low signal
    • Use of a “Pan-Flavivirus” RT-PCR and sequencing was consistent with Usutu
Emerging Virus Identification

• Viral Culture
  • Semi-unbiased approach to viral identification
  • Many emerging viruses cannot be cultured safely or efficiently
  • Maintenance of many cell lines is not practical or feasible for most clinical laboratories

• Next Generation Sequencing
  • Protocols for unbiased sequencing are available
  • Requires a highly sophisticate facility and resources for handling and interpreting large amounts of data
  • Confirmation of identified viruses as causative pathogens is difficult
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Conclusions

• Expect more DENV and CHIKV
  • Autochthonous transmission in the SE and regions with *A. albopictus*
  • Returning travelers: Americas, South & SE Asia

• Accurate, multiplex diagnostics for agents that cause a UFI should
  • Improve patient care
  • Decrease resource utilization
  • Aid in the detection of emerging pathogens by ruling out infections that are common in a given region.
Thank You