Clinical, Diagnostics and Therapeutics in Arbovirus Infections

David Speers
Arboviruses

**ALPHAVIRUSES**

Human Disease
- *Ross River virus*
- *Barmah Forest virus*
- *Sindbis virus, Chikungunya*

**FLAVIVIRUSES**

Human disease
- *MVE, Kunjin/West Nile virus, dengue*
- *Japanese encephalitis*
- *Kokobera, Yellow Fever, Zika, TBE*

**BUNYAVIRUSES**

Human Disease
- *Gan Gan, Hantavirus*
- *Trubanaman, Rift Valley Fever, Crimean-Congo haemorrhagic fever*

*Australian*
### Arbovirus Notifications in Australia, 2013/14

<table>
<thead>
<tr>
<th>Arbovirus</th>
<th>NSW</th>
<th>NT</th>
<th>Qld</th>
<th>SA</th>
<th>Vic</th>
<th>WA</th>
<th>TOTAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>RRV</td>
<td>509</td>
<td>434</td>
<td>1,845</td>
<td>111</td>
<td>161</td>
<td>1,485</td>
<td>4,569</td>
</tr>
<tr>
<td>BFV</td>
<td>254</td>
<td>129</td>
<td>1,115</td>
<td>20</td>
<td>25</td>
<td>257</td>
<td>1,803</td>
</tr>
<tr>
<td>Dengue*</td>
<td>211</td>
<td>69</td>
<td>461</td>
<td>82</td>
<td>414</td>
<td>531</td>
<td>2,021</td>
</tr>
<tr>
<td>MVE</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>CHIK^</td>
<td>22</td>
<td>2</td>
<td>8</td>
<td>5</td>
<td>20</td>
<td>37</td>
<td>94</td>
</tr>
<tr>
<td>Yellow F</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

*mostly imported cases (404 local Queensland cases)

^all imported cases

National Arbovirus and Malaria Advisory Committee
Clinical aspects of arboviruses

- Incubation period of 3 days to 2 weeks for most
- Most arbovirus infections are asymptomatic
- Ranges from mild febrile illness to severe encephalitis
- Acute symptom duration from 3-10 days
- Categorised into neuroinvasive and non-neuroinvasive
Illnesses due to Arboviruses

- **Non-neurological:**
  - Polyarthralgic illness: Ross River, Barmah Forest, Kunjin/West Nile virus, Kokobera, Gan Gan, Trubanaman, Chikungunya, Dengue
  - Fever and rash: Ross River, Barmah Forest, Dengue, Zika
  - Febrile illness: Dengue, MVE, Kunjin, JE
  - Haemorrhagic fever: Dengue, CCHF, Rift Valley Fever, Hantavirus, Yellow Fever

- **Neurological**
  - Meningitis/Encephalitis/Acute flaccid paralysis: MVE, Kunjin, JE, TBE, Rift Valley Fever
  - Guillain-Barre Syndrome: Zika
  - Congenital cerebral malformations: Zika
  - Ocular: Rift Valley Fever
Age distribution of cases of Ross River virus disease, south-west of WA, July 1995 - June 1996

(M:F = 1.1:1.0)
Ross River Virus Infection

- Arthralgia
- Arthritis
- Lethargy
- Myalgia
- Rash
- Fever

Bar chart showing the percent occurrence of various symptoms.
### Comparison of disease due to Barmah Forest virus with Ross River virus

<table>
<thead>
<tr>
<th>Symptom</th>
<th>BFV Disease</th>
<th>RRV Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rash</td>
<td>52 - 100%</td>
<td>40 - 60%</td>
</tr>
<tr>
<td>: Maculopapular</td>
<td>90%</td>
<td>100%</td>
</tr>
<tr>
<td>: Vesicular</td>
<td>10%</td>
<td>Uncommon</td>
</tr>
<tr>
<td>Joint swelling/stiffness</td>
<td>30%</td>
<td>61 - 80%</td>
</tr>
<tr>
<td>Joint pain : Any</td>
<td>70 - 86%</td>
<td>83 - 98%</td>
</tr>
<tr>
<td>: ≥ 1 month</td>
<td>40%</td>
<td>80 - 98%</td>
</tr>
<tr>
<td>: ≥ 6 months</td>
<td>≥ 10%</td>
<td>57%</td>
</tr>
<tr>
<td>Myalgia</td>
<td>70 - 80%</td>
<td>43 - 67%</td>
</tr>
<tr>
<td>Fatigue</td>
<td>80%</td>
<td>62 - 94%</td>
</tr>
<tr>
<td>Fever</td>
<td>50%</td>
<td>20 - 59%</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
<td>7%</td>
<td>0.6 - 20%</td>
</tr>
</tbody>
</table>
Murray Valley Encephalitis Virus

- **Presentation**
  - Altered mental state, seizures, tremor, weakness, paralysis

- **Blood tests**
  - Raised CRP, LFTs, neutrophils, platelets

- **EMG**
  - Diffuse slow wave pattern

- **Radiology**
  - Early CT head often normal
  - MRI:
    - Thalamic signal worse prognosis
    - Thalamic + brainstem, basal ganglia, cerebellum or cortex involvement devastating outcome
Murray Valley Encephalitis Virus
Dengue Virus

Primary infection
- Short incubation of < 9 days
- Fever, retro-orbital headache, myalgias, nausea
- Acute illness lasts 3-7 days, convalescence may last weeks

Dengue haemorrhagic fever
- Due to infection with a second serotype
  - Following classical dengue but when fever breaks develop haemorrhages, circulatory collapse, thrombocytopenia
  - If severe vascular leak develop dengue shock syndrome
Reported clinical symptoms among confirmed Zika cases

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>N (n=31)</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Macular or papular rash</td>
<td>28</td>
<td>90%</td>
</tr>
<tr>
<td>Subjective fever</td>
<td>20</td>
<td>65%</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>20</td>
<td>65%</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>17</td>
<td>55%</td>
</tr>
<tr>
<td>Myalgia</td>
<td>15</td>
<td>48%</td>
</tr>
<tr>
<td>Headache</td>
<td>14</td>
<td>45%</td>
</tr>
<tr>
<td>Retro-orbital pain</td>
<td>12</td>
<td>39%</td>
</tr>
<tr>
<td>Edema</td>
<td>6</td>
<td>19%</td>
</tr>
<tr>
<td>Vomiting</td>
<td>3</td>
<td>10%</td>
</tr>
</tbody>
</table>

Yap Island, 2007
Clinical features: Zika c.f. dengue and chikungunya

<table>
<thead>
<tr>
<th>Features</th>
<th>Zika</th>
<th>Dengue</th>
<th>Chikungunya</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fever</td>
<td>++</td>
<td>+++</td>
<td>+++</td>
</tr>
<tr>
<td>Rash</td>
<td>+++</td>
<td>+</td>
<td>++</td>
</tr>
<tr>
<td>Conjunctivitis</td>
<td>++</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>++</td>
<td>+</td>
<td>+++</td>
</tr>
<tr>
<td>Myalgia</td>
<td>+</td>
<td>++</td>
<td>+</td>
</tr>
<tr>
<td>Headache</td>
<td>+</td>
<td>++</td>
<td>++</td>
</tr>
<tr>
<td>Hemorrhage</td>
<td>-</td>
<td>++</td>
<td>-</td>
</tr>
<tr>
<td>Shock</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>

Rabe, Ingrid MBChB, MMed
“Zika Virus- What Clinicians Need to Know?” (presentation, Clinician Outreach and Communication Activity (COCA) Call, Atlanta, GA, January 26 2016)
Reported Zika neurological sequelae

- Guillain-Barré syndrome (GBS)
- Acute myelitis
- Meningoencephalitis
- Acute disseminated encephalomyelitis (ADEM)
- Sensory polyneuropathy
- Uveitis

French Polynesian outbreak
(Lancet 2016;387:1531)
Zika and pregnancy outcomes

(Congenital Zika Syndrome)

- Birth defects reported in 6% of maternal infections
  - No difference if maternal Sx (JAMA 2017;317:59)
- Miscarriage or stillbirth
- Microcephaly
  - esp 1st trimester infection
- Intracranial calcifications
- Absent or poorly developed brain structures
- Eye defects
  - esp 1st trimester infection
  - retinal scarring, optic nerve hypoplasia
- Hearing deficits (Brazil: 6% incidence)
- Limb contractures (arthrogryposis in 20%)
  - Due to brain stem, spinal cord abnormalities
- IUGR
Zika effects on human neurons

- Zika has tropism for:
  - neural stem cells
    - apoptosis (cell death)
  - radial glial cells
    - straddle base to surface of brain to provide scaffold used to populate cortex

- Induces increased centrosomes in foetal brain cells
  - associated with failure of cell division and microcephaly

- Result in:
  - insufficient neurons producing microcephaly
  - congenital cortical malformation (scaffolding defect)
Arbovirus Testing

- Combination of:
  - direct detection
  - serological methods

- Laboratory methods need to be considered together with other information:
  - vaccination and travel history
  - date of onset of symptoms
  - other arboviruses known to circulate in the geographic area
Direct detection methods

- **NAAT:**
  - blood (whole blood vs serum), urine, vaginal fluid, semen
  - arboviruses often detectable in serum for only the first few days of illness prior to appearance of antibody

- **Antigen detection:**
  - NS1 Ag for DENV

- **Culture:**
  - requires insect cell lines for 3-4 days then passaged into a mammalian cell line for another 2-3 days for a CPE
  - Previously suckling mouse brain or intrathoracic mosquito inoculation
NAAT detection

- Commercial assays available, many in-house assays

- Sensitivity peaks usually within 2-3 days in serum then declines rapidly through first week of illness
  - Can be serotype (DENV), lineage (CHIK, ZKV) variation in sensitivity

- Detection often dependent on body fluid

- Can also genotype, perform phylogenetic studies:
  - DENV 1-4
  - Zika virus Asian vs African lineage
Detection of Zika Virus by specimen

- **Serum:**
  - Up to 7 days
  - Case reports of prolonged viraemia in pregnancy due to foetal infection (NEJM 2016)

- **Urine:**
  - Up to 14 days

- **Semen:**
  - Detected by PCR up to 6 months after symptom onset, cleared by 3 months in 95%
  - Zika cultured from semen up to 93 days
  - Testicular atrophy, infertility reported in mouse model (Nature 2016)

- **Vaginal fluids**
  - PCR detection up to 14 days,

- **Breast milk**
  - RNA has been detected in breast milk.
  - Zika virus cultured from breast milk in one report (Lancet 2016;387(10023):1051)
  - No reports of transmission through breastfeeding.

- **Eye**
  - Up to 1 week
ZKV detection by PCR

- 150 Zika cases
  Paz-Bailey et al., NEJM 2017
- single Zika case
  - Alpha/Flavivurses known to agglutinate some animal RBCs
  - WNV known to adhere to human RBCs (Rios et al., CID)
  Murray et al., EID 2016
Arbovirus serological assays

- **Serology**
  - Serum, CSF

- **Assays for the detection of IgM and IgG antibodies include:**
  - enzyme immunoassay (EIA)
  - microsphere immunoassay (MIA)
  - haemagglutination inhibition assay (HI)
  - immunofluorescence assay (IFA).

- **These assays provide a presumptive diagnosis**
  - Need confirmatory testing in some circumstances, e.g. dengue IgM, NS1 Ag detection in a non-traveller
  - Confirmatory testing includes plaque reduction neutralization test (PRNT), other neutralisation platforms, monoclonal blocking EIAs.
Haemagglutination inhibition

- Arboviruses naturally agglutinate goose RBCs, some mammalian RBCs
- May need to use virus-specific antigen
Plaque reduction neutralisation Titre (PRNT)

- Gold standard for arboviruses, other viruses, e.g. mumps
- Very laborious and difficult but measures virus-specific neutralising antibodies
- Must have a comparator virus
Interpreting arboviral laboratory serology results (1)

- **Rise and fall of IgM antibodies:**
  - IgM antibodies generally first detectable at 3-8 days after onset of illness and persist for 30-90 days

- **Persistence of IgM antibodies:**
  - Arboviral IgM antibodies may be detected in some patients months or years after their acute infection. e.g., up to 500 days for West Nile virus
  - Virus-specific IgM antibodies in CSF, fourfold or greater rise in virus-specific antibody titres between acute- and convalescent-phase serum specimens more reliable.

- **Persistence of IgG and neutralizing antibodies:**
  - Arboviral IgG and neutralizing antibodies can persist for many years.
  - The presence of these antibodies alone is only evidence of infection at some time
Serologic cross-reactivity:
- Arboviruses from the same genus produce cross-reactive IgM and IgG antibodies.
- Case report of Zika virus infection causing false positive dengue NS1 Ag
  - Used SD Dengue Duo NS1 Ag device (Swiss Med Wkly 2016;146)
  - Platelia Dengue NS1 Ag kit not found to cross-react in 65 acute ZKV cases (EID 2016;22:1692)

In regions where two or more closely-related arboviruses occur, problem of ‘Original antigenic sin’
- React to past flavivirus infection before reacting to current flavivirus infection
  - Higher titre to past virus infection or false negative for recent infection
- Problem with past infection or vaccination
  - Previous Dengue infection or YF vaccination and recent Zika
  - Recent KUNV/MVEV and past MVEV/KUNV
Serology profile depends on primary vs secondary infection

Primary DENV Infection

Secondary DENV Infection
New testing modalities

- Microfluidics advances:
  - Can test saliva, blood, urine
  - Little skill required
  - Cheap, rapid TAT
  - Suitable as POCT
  - Being adapted to ZIKV detection
Arbovirus treatment

- No specific antivirals available
- Symptomatic treatment
  - Rest
  - Drink fluids to prevent dehydration
  - Paracetamol to reduce fever and pain
    - Avoid NSAIDs and aspirin in DNV infection
- Experimental therapies for flaviviruses
  - Neutralising antibody
    - Ab to envelope, NS1 flavivirus proteins
    - Zika antibody therapy protected foetus in mouse model
  - Repurposing existing compounds
  - Antiviral development (hepatitis C in Flaviviridae family)
Flavivirus genome
Flavivirus replication
Existing drugs

- Nitazoxanide
  - Antiparasitic drug for Giardia
  - Active against JEV, DENV-2 and YFV in cell culture
  - Active against JEV in mouse model

- Bromocriptine
  - Dopamine agonist
  - Active against DENV 1-4, TBEV in focus reduction assays

- Ivermectin
  - Broad-spectrum antiparasitic drug
  - Active against DENV 1-4, ZIKV
Nature Reviews / Drug Discovery
– online 5 May 2017 doi:10.1038/nrd.2017.33

Broad-spectrum agents for flaviviral infections: dengue, Zika and beyond

Veaceslav Boldescu, Mira A. M. Behnam, Nikos Vasilakis and Christian D. Klein
Arbovirus (flavivirus) human vaccines

- Vaccines available in Australia
  - JEV: live and inactivated
    - Some animal model evidence for cross protective antibody response to MVEV
  - Yellow Fever: live 17D virus
    - Travel requirements, current shortage (African, Brazil outbreaks and supply problem)
    - WHO recommending 1/5 dose in outbreaks

- Vaccines available overseas
  - Dengue: live attenuated tetravalent
    - Hindered by concerns of immune enhancement
    - Dengvaxia®
  - TBE: inactivated
    - Can access through travel clinics in Aust via SAS
Zika vaccines

- 40 vaccine candidates:
  - 5 entering phase 1 studies by Feb 2017
    - Inactivated, mRNA, DNA vaccines
  - 2-3 years for registration, expected 2020

Rubella: The last virus to cause an epidemic of congenital defects
WHO agenda for arbovirus vaccines

- **Area 1 (Dengue):**
  - development of second generation dengue vaccines
  - dengue diagnostic algorithms post dengue vaccine introduction

- **Area 2 (Zika):**
  - analyse Zika vaccines, diagnostics and therapeutics
  - define strategic priorities

- **Area 3 (Yellow fever):**
  - Yellow fever vaccine fractional dose agenda

- **Area 4 (Arboviruses in general):**
  - advance the arboviral vaccine development agenda
**Novel vaccine strategies**

- **Vaccines in animals/development/experimental**
  - Killed RRV (Wressnigg et al 2015 Clin Vacc Immunol)
  - CHIK (Metz et al 2013 PLoS Negl Trop Dis)
  - WNV DNA equine vaccine
  - MVE mouse model (Hall et al 1996, J Gen Virol)
  - Rift Velley Fever

- **Baculovirus vaccines**
  - Insect virus non-pathogenic in humans
  - Express other enveloped virus glycoproteins correctly and at high level

- **Transmission blocking vaccines**
  - Stop mosquito infection rather than human infection
Summary

- Clinical
  - Non-neurological
    - Fever,
    - Fever and rash
    - Fever, rash arthralgia
  - Neurological
    - Encephalitis
    - Congenital cerebral malformation
- Laboratory diagnosis of arboviruses complicated by:
  - High rate of asymptomatic cases
  - Serological cross-reactivity
  - Brief early viraemia in many arbovirus infections limiting direct detection
- Treatment
  - Symptomatic at present, novel inhibitors in the future