West Nile and Dengue virus

Mical Paul
Rambam Health Care Center
Tel-Aviv University
Israel
Flaviviruses
West Nile Virus
“Arbovirus” (arthropod-borne virus) that is transmitted by a mosquito vector

WNV: Culex sp.
Transmission cycle

- *Culex* mosquitoes are the primary global transmission vector
- Cycle between the mosquito, animal host (most commonly birds) and humans as dead-end hosts
  - *Culex* mosquitoes feed on birds and humans (shift during summer months)
- Currently no transmission between humans
  - Potentially possible if Aedes mosquitoes, which feed primarily on humans, become primary transmission vectors for WNV
- Rare modes of transmission: blood transfusion, organ transplantation, transplacental infection, breastfeeding, and laboratory-acquired infection
The poor birds

- Birds may have viremia that lasts for more than 100 days
- Highest titer viremias have been reported in jays, grackles, finches, crows, sparrows
  - transmission to more than 80% of biting mosquitoes
- In 1998, a change has been observed in Israel, with birds starting to die of WNV
- Sudden die-off of crows or other passerine species can serve as a sentinel event presaging subsequent human epidemics
  - might explain why human cases often decrease sharply in a particular year after a major WNV outbreak
Epidemiology
Clinical features

- Asymptomatic: 80 cases
- West Nile fever: 19 cases
- Neuroinvasive disease: 1 case
Clinical manifestations - WNF

- Fever (abrupt onset)
- Headache
- Fatigue, with variable malaise, anorexia, nausea,
- Myalgia
- Lymphadenopathy
- Nonpruritic generalized maculopapular rash (rare)
Neuro-invasive WNV disease

- Meningitis ~40%
- Encephalitis ~60%
  - Myoclonus
  - Visual problems (fundoscopic examination)
- Acute flaccid paralysis /poliomyelitis ~5-10%
  - Asymmetric
  - Younger patients

Usually features are combined
Risk factors for neurological complications

• Older age
  – Age >70 yrs. case-fatality rate ranges from 15% to 29%

• Immunosuppression
  – Solid organ transplantation

• Alcohol abuse

• Diabetes

• Chronic renal disease

• CCR5 deficiency (associated with decreased for HIV infection after exposure)
Diagnosis

• Viremia within 1 to 2 days of a mosquito bite, typically persists for up to a week
  – Low level, difficult to detect
  – Usually absent at the time of symptomatic illness
  – Blood product screening performed through nucleic acid testing of plasma (US)
• IgM seroconversion is observed as viremia ends (may persist for >2 months)
• IgG seroconversion ~ 8 days later (remains elevated for a long time after infection >1 year)
• IgA appears in serum in between IgM and IgG
CSF features

• Mild pleocytosis, mean cell count 227/mm³, predominantly lymphocytic (~60%)
• Normal glucose
• Elevated protein: 76mg/dl in meningitis and 101mg/dl in encephalitis
Diagnosis of neuroinvasive disease

- WNV IgG can cross the blood–brain barrier, thus its presence in the CSF is not diagnostic
- WNV IgM is produced in-situ in CSF during WNV encephalitis, thus its detection in CSF is diagnostic for WNV encephalitis
  - WNV IgM can persist in CSF for up to 199 days
- IgA in CSF is also specific for WNV encephalitis
- Identification of WNV in CSF
  - viral culture (biosafety!)
  - WNV polymerase chain reaction (sensitivity 57-70% compared to WNV IgM)
Test interpretation

<table>
<thead>
<tr>
<th># Paired samples</th>
<th>WNV IgA, IgG and IgM qualitative reaction patterns for 139 paired CSF and serum samples collected during the 2005 season</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CSF</td>
</tr>
<tr>
<td></td>
<td>IgA</td>
</tr>
<tr>
<td>119</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td>1</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
</tr>
</tbody>
</table>

False positive WNV serology

- Cross-reacting antibodies with other Flaviruses
  - St Louis encephalitis
  - Japanese encephalitis
  - Dengue serotype 2
  - Yellow fever
  - Powassan viruses
- Rheumatoid arthritis/ other autoimmune for IgM
Suggested diagnostic criteria

- Fever AND
- Acute systemic signs AND
- Neurological symptoms/ signs/ CSF abnormality with no alternative diagnosis AND
- at least one of:
  A. Demonstration of WNV IgM antibody in serum without vaccination with yellow fever or Japanese B viral vaccines in past 5 years or recent infection with other flavivirus, such as St Louis encephalitis virus. Note: If WNV has occurred in region in prior years, criterion B is needed because previously infected individuals may have prolonged persistence of IgM in serum.
  B. Fourfold or greater increase in serum WNV IgG or IgM antibody titer between acute and convalescent samples taken 10 to 28 days apart.
  C. Demonstration of WNV IgM antibody in CSF
  D. Identification of WNV in CSF by viral culture or of WNV nucleic acid by polymerase chain reaction
Brain imaging

- CT normal
- MRI usually normal (20 to 70% of patients with WNV neurological disease)
  - Observed more early-on
  - When present, predilection for deep gray matter structures including the basal ganglia, thalami, brainstem and cerebellum
MRI neuroimaging WNV

Thalamus

Substantia nigra
Neurological recovery

- Prolonged!
- Age is the most important risk factor for adverse long-term outcomes
- New York City outbreak in 1999: only 37% had achieved a full recovery at 1 year after illness
  - Cognitive impairments: memory loss (44%), depression (44%), irritability (39%), lightheadedness (37%), loss of concentration (33%), confusion (31%)
- Recovery unrelated to severity of initial encephalitis
Treatment

- No proven antiviral
- Interferon alpha suggested, not proven, no RCTs
- Ribavirin reported as associated with worst outcome
- IVIG containing high titers of WNV IgG was associated with variable improvement in outcomes in case reports and case series in Israel
  - RCTs of IVIG or monoclonal antibodies terminated due to slow accrual
Prevention

- Reduction in risk for exposure to infected mosquitoes
  - Personal
  - Public
- Human vaccine: live attenuated vaccine in phase II trials, proven safe and immunogenic in healthy adults and the elderly (ChimeriVax-WN02, Sanofi Pasteur)
  - Chimeric vaccine: produced by insertion of the genes encoding the pre-membrane and envelope E proteins of WNV (strain NY99) into the yellow fever vaccine clone., with the E gene mutated at 3 sites to reduce
Vaccine trial outcome measurements

- Immunogenicity: plaque reduction neutralization test to determine the neutralizing antibody levels (ChimeriVax-WN02 vaccine virus and OrVax-Vero cells). Examined at day 0 and 28 post-injection and defined as four-fold or greater rise in titer.
- IgM antibodies: measured qualitatively by ELISA.
- Viremia: analysed for a subset of patients. Blood taken every second day. Plaque assay on duplicate Vero cell monolayers. Detectable at ≥20 pfu/mL and <60 pfu/mL and quantifiable at ≥60 pfu/mL.
Viremia followed by seroconversion rates at day 28

Viremia followed by seroconversion rates at day 28

Dayan et al. Vaccine 2012; 30: 6656–6664. Phase II, double blind, dose ranging, >50 years

<table>
<thead>
<tr>
<th>Summary of viremia.</th>
<th>WN02 4 × 10³ (A)</th>
<th>WN02 4 × 10⁴ (B)</th>
<th>WN02 4 × 10⁵ (C)</th>
<th>Placebo</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 80</td>
<td>N = 82</td>
<td>N = 73</td>
<td>N = 80</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Log10 (Cmax in pfu/mL)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M Mean [95% CI]</td>
<td>49.16 [1.55; 1.72]</td>
<td>50.17 [1.63; 1.84]</td>
<td>45.17 [1.61; 1.81]</td>
<td>1.04 [NC]</td>
<td>0.398</td>
</tr>
<tr>
<td>Duration (days)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects with detectable viremia 20 pfu/mL and &lt;60 pfu/mL</td>
<td>34.253 [1.80; 3.26]</td>
<td>33.270 [1.88; 3.51]</td>
<td>26.245 [1.66; 3.23]</td>
<td>0</td>
<td>0.969</td>
</tr>
<tr>
<td>Subjects with quantified viremia 60 pfu/mL</td>
<td>15.593 [3.93; 7.94]</td>
<td>17.524 [3.79; 8.68]</td>
<td>16.400 [2.60; 3.40]</td>
<td>1.100 [NC]</td>
<td>0.203</td>
</tr>
<tr>
<td>Days viremic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Subjects with detectable viremia 20 pfu/mL and &lt;60 pfu/mL</td>
<td>34.153 [1.28; 1.78]</td>
<td>32.162 [1.31; 1.93]</td>
<td>0</td>
<td>0.927</td>
<td></td>
</tr>
<tr>
<td>Subjects with quantified viremia 60 pfu/mL</td>
<td>15.307 [2.14; 3.99]</td>
<td>17.288 [2.18; 3.95]</td>
<td>16.225 [1.68; 2.82]</td>
<td>1.100 [NC]</td>
<td>0.282</td>
</tr>
</tbody>
</table>

Geometric mean titer and seroconversion rates by plaque reduction neutralization test

<table>
<thead>
<tr>
<th>Summary of titers and seroconversion rates by plaque reduction neutralization test.</th>
<th>WN02 4 × 10³ (A)</th>
<th>WN02 4 × 10⁴ (B)</th>
<th>WN02 4 × 10⁵ (C)</th>
<th>Placebo</th>
<th>P-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>N = 114</td>
<td>N = 108</td>
<td>N = 114</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Day 0 (pre-vaccination)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Geometric mean titer [95% CI]</td>
<td>5.00 [NC]</td>
<td>5.76 [4.92; 6.74]</td>
<td>5.06 [4.94; 5.20]</td>
<td>5.22 [4.80; 5.68]</td>
<td>0.085</td>
</tr>
<tr>
<td>M/N [%], [95% CI]</td>
<td>688 [453; 1047]</td>
<td>600 [405; 890]</td>
<td>674 [464; 978]</td>
<td>5.93 [4.96; 7.08]</td>
<td>0.870</td>
</tr>
<tr>
<td>Seroconversion &lt;1:10 at baseline and 1:20 on Day 28</td>
<td>105/114 [92.1; 95.5; 96.3]</td>
<td>110/118 [93.2; 97.1; 97.0]</td>
<td>103/108 [95.4; 95.9; 98.5]</td>
<td>3/114 [2.6; NC]</td>
<td>0.749</td>
</tr>
<tr>
<td>Seroconversion ≥1:10 at baseline and fourfold on Day 28</td>
<td>105/114 [92.1; 95.5; 96.3]</td>
<td>109/118 [92.4; 98.0; 99.5]</td>
<td>102/108 [94.4; 98.3; 99.7]</td>
<td>3/114 [2.6; NC]</td>
<td>0.749</td>
</tr>
<tr>
<td>P-values</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>0.085</td>
<td>0.048</td>
<td>0.308</td>
<td>0.207</td>
<td></td>
</tr>
<tr>
<td>A vs. B</td>
<td>0.870</td>
<td>0.623</td>
<td>0.940</td>
<td>0.683</td>
<td></td>
</tr>
<tr>
<td>A vs. C</td>
<td>0.400</td>
<td>0.596</td>
<td>0.600</td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>B vs. C</td>
<td>0.767</td>
<td>1.000</td>
<td>0.485</td>
<td>1.000</td>
<td></td>
</tr>
</tbody>
</table>
Dengue virus

Aedes aegypti
Virology

- Four antigenically distinct serotypes (DENV 1-4)
- Infection by a specific serotype confers lifelong immunity against the specific serotype but not to the remaining three
- Antibody dependent enhancement (ADE): an increased risk for severe disease (dengue hemorrhagic fever) with secondary infection of a different serotype. Binding of crossreactive, neutralizing antibodies at subneutralizing concentration that enhances the infection of monocytes and dendritic cells
- Epidemics of severe disease are usually reported from areas where two or more serotypes circulate in succession or simultaneously.
Epidemiology

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

*countries or areas at risk (As of 1 November 2008)*
Figure 1.2 Average annual number of dengue fever (DF) and dengue haemorrhagic fever (DHF) cases reported to WHO, and of countries reporting dengue, 1955–2007.
Recent outbreaks

Travel-related dengue infections acquired in Luanda, Angola, reported from GeoSentinel sites, March–May 2013 (n=10)

<table>
<thead>
<tr>
<th>Country of origin of the case</th>
<th>Fever onset date</th>
<th>Time from fever onset to test (days)</th>
<th>NS1</th>
<th>Serology-IgM</th>
<th>Qt-PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Germany</td>
<td>30 March</td>
<td>4</td>
<td>Positive</td>
<td>Positive</td>
<td>ND</td>
</tr>
<tr>
<td>Canada</td>
<td>3 April</td>
<td>10</td>
<td>Negative</td>
<td>Positive</td>
<td>ND</td>
</tr>
<tr>
<td>France</td>
<td>5 April</td>
<td>12</td>
<td>Negative</td>
<td>Positive</td>
<td>ND</td>
</tr>
<tr>
<td>Germany</td>
<td>7 April</td>
<td>14</td>
<td>Negative</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>South Africa</td>
<td>10 April</td>
<td>7</td>
<td>ND</td>
<td>Positive</td>
<td>Negative</td>
</tr>
<tr>
<td>Israel</td>
<td>11 April</td>
<td>14</td>
<td>ND</td>
<td>Positive</td>
<td>ND</td>
</tr>
<tr>
<td>Israel</td>
<td>17 April</td>
<td>7</td>
<td>Positive</td>
<td>Positive</td>
<td>ND</td>
</tr>
<tr>
<td>Israel</td>
<td>18 April</td>
<td>4</td>
<td>Positive</td>
<td>Positive</td>
<td>DENV-1</td>
</tr>
<tr>
<td>Israel</td>
<td>25 April</td>
<td>5</td>
<td>Positive</td>
<td>Positive</td>
<td>DENV-1</td>
</tr>
<tr>
<td>Israel</td>
<td>2 May</td>
<td>6</td>
<td>Positive</td>
<td>Positive</td>
<td>ND</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>DenOFIs</th>
<th>p-value</th>
<th>Children (&lt;15 years)/Adults (&gt;15 years)</th>
<th>p-value</th>
</tr>
</thead>
</table>
| Nausea                | 50.0|28.9%  
58.0|49.0%†  
51.3|30.5% | <0.00001  
<0.05  
<0.001 | 50.2|76.4%  
58.2|76.4% | <0.001 |
| Vomiting              | 16.4|8.4%   
16.2|8.5%  
57.0–64.0|31.0–46.0%†  
70.0|52.0%† | <0.00001  
0.03  
<0.01  
<0.05 | 50.2|76.4%  
58.2|76.4% | <0.001 |
| Retro-orbital pain    | 26.0|15.9%   
26.6|13.5%  
10.01‡  | <0.00001  
0.003  
0.001 | 8.7|29.1%  
<0.001 |
| Aches/pains           | 1.4§  | <0.00001 | 20.3|36.4% | 0.012 |
| Rash                  | 11.2–41.2|30.0–6.4% | <0.003/0.007 | NA | NA |
| Tourniquet test positive | 34.0|19.0%   
42.0|6.0%†  
43.0–65.0|21.0–29.0%  
1.86§ | 0.02  
<0.01  
<0.1  
<0.001 | NA | NA |
| Leukopenia            | $3.8 \times 10^3$|7.3 $\times 10^3$/µl   
$4.5 \times 10^3$|8.1 $\times 10^3$/µl  
<4.5 $\times 10^3$/µl: 72.1|11.5% | <0.00001  
<0.1  
<0.001 | NA | NA |
| Thrombocytopenia (platelet/mm$^3$) | 16|4% (≤100,000)†  
16|82%† (≤100,000)  
66|95%† (≤100,000)  
14.9|1.5% (≤100,000)  
32,000|96,500  
163,500|239,000  
70,000|104,000* | NA | NA | NA |
Tourniquet test
WHO suggested dengue case classification and levels of severity

### Dengue ± Warning Signs

- With warning signs
- Without warning signs

### Severe Dengue

1. Severe plasma leakage
2. Severe haemorrhage
3. Severe organ impairment

### Criteria for Dengue ± Warning Signs

- **Probable dengue**
  - Live in/travel to dengue endemic area.
  - Fever and 2 of the following criteria:
    - Nausea, vomiting
    - Rash
    - Aches and pains
    - Tourniquet test positive
    - Leukopenia
    - Any warning sign

- **Laboratory-confirmed dengue**
  (important when no sign of plasma leakage)

- **Warning signs**
  - Abdominal pain or tenderness
  - Persistent vomiting
  - Clinical fluid accumulation
  - Mucosal bleed
  - Lethargy, restlessness
  - Liver enlargement >2 cm
  - Laboratory: increase in HCT concurrent with rapid decrease in platelet count

### Criteria for Severe Dengue

- **Severe plasma leakage leading to:**
  - Shock (DSS)
  - Fluid accumulation with respiratory distress

- **Severe bleeding**
  as evaluated by clinician

- **Severe organ involvement**
  - Liver: AST or ALT $\geq 1000$
  - CNS: Impaired consciousness
  - Heart and other organs
Dengue diagnosis
# Serology interpretation

<table>
<thead>
<tr>
<th>Diagnosis of Dengue</th>
<th>Serology Results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highly suggestive</td>
<td>Positive IgM in single serum sample</td>
</tr>
<tr>
<td></td>
<td>Positive IgG in a single sample with an HI titer of $\geq 1280$</td>
</tr>
<tr>
<td>Confirmed diagnosis</td>
<td>IgM seroconversion in paired sera</td>
</tr>
<tr>
<td></td>
<td>IgG seroconversion in paired sera or $\geq 4$-fold IgG titer in paired sera</td>
</tr>
<tr>
<td>Primary infection</td>
<td>Negative IgG in the acute-phase serum and a positive IgG in the convalescent-phase serum</td>
</tr>
<tr>
<td></td>
<td>Ratio of IgM and IgG in single serum sample $\geq 1.2$</td>
</tr>
<tr>
<td>Secondary infection</td>
<td>Positive IgG in the acute-phase serum and a $4$-fold rise in IgG titer in the convalescent-phase serum sample</td>
</tr>
<tr>
<td></td>
<td>Ratio of IgM and IgG in single serum sample $\leq 1.2$</td>
</tr>
</tbody>
</table>
Dengue virus vaccine

• Must be equally protective against each of the four DENV serotypes to reduce the risk of ADE
• Vaccine candidates under development
  – live attenuated virus
  – live chimeric virus most advanced (Sanofi Pasteur ChimeriVax Dengue tetravalent vaccine). Low and unequal effectiveness (~30%)
  – inactivated virus
  – live recombinant DNA
  – subunit vaccines
• No licensed vaccine to date. The future...
Recommended reading