Diagnosis of Varicella Zoster Virus Infection

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Varicella Zoster Virus: Virology

- 150-200nm typical herpes particle
- Icosahedral capsid 162 capsomeres
- Ds linear DNA 125 kb, 68 ORFs
- Lipid envelope with glycoprotein spikes
- Single serotype
- Replication largely restricted to primates
- Labile, slow growing \textit{in vitro}

Varicella Zoster Virus: Epidemiology

- Endemic
- Winter/early spring seasonality
- Primary infection mostly children < 10 years
- 95% adults seropositive, (75% no VZ history immune)
- 2° household attack rate 60-90%


Deaths/100,000 Cases

Varicella: Pathogenesis

- Respiratory transmission
- Transmission 4 days before rash to 5 days after
- 10-21 day incubation period
- Fenners mousepox model
- Less cell destructive than HSV
- CMI important to immunity
- Latency in sensory nerve ganglia

Varicella: Clinical Presentation

- Several days flu-like prodrome in older children/adults
- Cropping rash trunk/scalp to periphery
- Evolves macules-vesicles-crusts over 8-12 hr
- Mild fever, headache malaise
- Increased severity in adults/immunosuppressed
- Complications: skin infection, CNS involvement, pneumonitis
- Severe neonatal disease if mother infected 2 days before – 4 days after birth
Zoster: Pathogenesis and Clinical Presentation

- Incidence 10-20%
- Declining CMI and humoral immunity increase risk
- Virions travel down sensory neuron to skin
- Prodrome of pain/paraesthesia 1-4 days
- Unilateral vesicular rash confined to a sensory dermatome usually thoracic or trigeminal nerve (ophthalmic)
- Complications
  * dissemination in immunosuppressed
  * motor neuropathy
  * pneumonitis
  * post-herpetic neuralgia

Whitley RJ. In: Mandell, Douglas and Bennett’s Principles and Practice of Infectious Diseases 6th Ed, 2005.

Varicella Zoster Complications

Skin infection
- Secondary bacterial infection of vesicles
- Most common VZV complication

CNS infection
- Acute cerebellar ataxia 1/4000 cases < 15 years
- Encephalitis 0-2% cases

Pneumonitis
- Potentially life threatening specially in pregnant women
- 0.25% cases

Past-herpetic neuralgia
- Chronic pain occurring in 25-50% patients over 50 years

Congenital Varicella Syndrome
- Occurs in <5% infants born to mothers with primary varicella, usually 1st trimester
- Cicatricial skin lesions, limb deformities, ocular abnormalities, CNS disease
Varicella zoster in the immunosuppressed

- Impaired CMI increases risk of severe disease
- HIV, malignancies esp haematological, esp. BMT
- Varicella may disseminate and/or recur
  - Extensive/prolonged skin lesions
  - Pneumonia
  - Hepatitis
  - Encephalitis
- Zoster may disseminate and/or recur
  - Extensive/prolonged skin lesions
  - Pneumonia
  - Encephalitis
  - Necrotizing retinopathy
Perinatal Varicella

• Maternal chickenpox around delivery may be life threatening for baby
• Baby’s illness severity relates to timing of maternal illness
• Worst outcome if baby infected transplacentally, then born before maternal IgG develops
• Baby IM ZIG candidate if:

  maternal chickenpox 5 days before to 2 days after delivery

<table>
<thead>
<tr>
<th>Timing of chickenpox in mother and baby in relation to severity</th>
<th>No died</th>
<th>No survived</th>
<th>Neonatal mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Onset of mother’s rash in relation to delivery</td>
<td>Onset of baby’s rash in relation to delivery (days)</td>
<td>0</td>
<td>27</td>
</tr>
<tr>
<td>5-21 days before</td>
<td>4 days before to 2 days after</td>
<td>0-4</td>
<td>5-10</td>
</tr>
</tbody>
</table>

Clinical diagnosis suffices for most varicella and zoster cases.

Laboratory diagnosis appropriate
- for patient management:
  - Immunosuppressed patient
  - Varicella in a neonate
  - Zoster in a young person
  - Atypical/unusual clinical presentations
  - Significant clinical complications
- for infection control/public health:
  - Index case in a nosocomial exposure event
  - Differentiation from smallpox in very selected cases
Varicella Zoster: Specimen Collection and Transport

Detection of virus:
- Vesicle fluid/infected cells from lesion base
- Suitable for IFA, EM, PCR, viral culture
- Deroofing fresh lesion and firm swabbing
- Viral transport medium
- Labile in transport
- Variable recovery from CSF, plasma, urine, t/s

Serology:
- Acute phase clotted blood
Varicella Zoster: Viral Culture

Cytopathic effect (CPE) in HDF Cell Culture

Uninfected

3 days post inoculation

6 days post inoculation

TUBE CULTURE
- Labile virus, transport delay further compromises recovery
- Grows only in human diploid and monkey kidney cell lines
- Approximately 1 week to positivity
- Characteristic CPE
- Confirmation usually by scraping and IFA

RAPID CULTURE
- Fibroblast cultures on coverslips or in multiwell trays
- Acetone fixation several days after inoculation
- Staining with FITC conjugated anti-VZV glycoprotein mab
- More sensitive/rapid than conventional tube culture

Varicella Zoster: Immunofluorescent (IFA) Antigen Detection

- Direct staining of vesicular cell smears with FITC mabs to gp98-gp62
- Rapid, result available in hours
- More sensitive than conventional tube cell culture (97.5 vs 49.4%)
- Critically depends on specimen quality (numbers of infected cells)
- Low throughput
- Subjective: requires skilled staff
Varicella Zoster: Electron Microscopy

- Typical herpes virions readily visualised from vesicle fluid
- Negative staining with PTA or UA
- Rapid but labour-intensive
- Role limited to differential diagnosis of smallpox in highly selected cases
Varicella Zoster: Nucleic Acid Testing

- Assay of choice for detection of VZV
- Many gene targets used for PCR: pol, IE etc
- Analytical sensitivity of 1-10 target copies possible
- Variety of assay formats - nested PCR and electrophoresis
  - real time SYBR green or probes
- Can multiplex with other viruses (HSV)
- Rapid: 6 hrs (nested) – 2.5 hrs (real time)
- Clinically sensitive and specific: CSF, ocular fluid, vesicle fluid, blood
Nucleic Acid Testing for VZV

- Nested multiplex PCR for 4 herpes viruses: VZV, HSV-1, HSV-2, CMV
Varicella Zoster: comparative sensitivity of diagnostic techniques from skin lesions

- 100 VZV infected patients
- 95 (95%) PCR
- 82 (82%) IFA
- 48 (48%) serology
- 20 (20%) culture

- 132 vesicular lesions
- 53 VZV PCR pos, 64 HSV PCR pos
- Relative sensitivities:
  - 100% PCR
  - 60% EM
  - 47% culture

- 253 dermal samples
- 44 (17%) PCR (real time, 2 primer sets)
- 23 (9%) shell vial MRC-5

- 110 dermal samples
- 51 (46%) PCR (nested and real time)
- 15 (14%) culture
NAT testing in assorted Varicella Zoster Complications

CNS Infection
- VZV DNA detected in CSF of 10/12 VZV infected patients with CNS involvement
- 7/7 zoster patients with CNS symptoms PCR pos
- 3/5 post chicken pox cerebellitis patients PCR pos
- 2/5 PCR neg were high dose acyclovir treated

Pneumonitis
- VZV DNA detection by PCR in BAL

Post-herpetic neuralgia
- VZV DNA detected by PCR in PBMC 11/51 PHN patients
- VZV DNA detected by PCR in PBMC 019 zoster patients
- Role of VZV persistence?

Congenital varicella syndrome
- 107 amniotic fluids from VZ infected pregnant women before 24/40
- 9/107 (8.4%) VZ PCR pos, 2/107 (1.8%) VZ culture pos
- 3/107 (2.8%) congenital varicella cases
### PCR for VZV DNA in Human Vitreous

<table>
<thead>
<tr>
<th>Clinical Diagnosis</th>
<th>No. of cases</th>
<th>No. Positive for VZV DNA</th>
</tr>
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<tbody>
<tr>
<td>HIV positive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VZV retinitis (PORN)</td>
<td>14</td>
<td>11</td>
</tr>
<tr>
<td>CMV retinitis</td>
<td>72</td>
<td>1</td>
</tr>
<tr>
<td>Other retinitis (CD4 count, [is less than] 1 00/[mm.sup.3])</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presumed HIV negative</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vitreoretinal inflammation</td>
<td>23</td>
<td>0</td>
</tr>
<tr>
<td>Vitreous hemorrhage</td>
<td>14</td>
<td>0</td>
</tr>
<tr>
<td>Macular hole/epiretinal membrane/retinal detachment</td>
<td>38</td>
<td>2</td>
</tr>
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Varicella Zoster: Virus Load in the Blood

Viral loads and days after onset in patients with varicella.

Comparison of viral loads in PBMC between acyclovir-treated and untreated groups

<table>
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<tr>
<th>Treatment</th>
<th>No. of patients</th>
<th>Days after onset (mean ± SEM)</th>
<th>No. of VZV genomes (mean ± SEM/10^5 cells) determined with:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acomlovir</td>
<td>5</td>
<td>4.0 ± 0.3</td>
<td>gb gene 7 ± 7^a ORF62 5 ± 5^a</td>
</tr>
<tr>
<td>None (untreated)</td>
<td>10</td>
<td>3.7 ± 0.3</td>
<td>gb gene 510 ± 354^b ORF62 382 ± 339^c</td>
</tr>
</tbody>
</table>


Quantitation of VZV DNA in vesicles and PBMC. (A) Vesicle fluid obtained from 3 patients with zoster or varicella. (B) PBMC obtained from 10 patients with zoster and 19 patients with varicella.

Q-PCR of seven consecutive plasma specimens from a patient who progressed from unidermatomal herpes zoster.
Varicella Zoster Virus: NAT in visceral zoster

- Disseminated VZV may present with severe abdominal pain before/without rash
- Especially in bone marrow transplant
- Grave outcome if diagnosed and treated as GVHD
- VZV retrospectively shown present in blood prior to rash in 4 subjects
- VZV present in blood/faeces after rash

(de Jong et al 2001) Lancet 357 p2101-2102
Varicella Zoster: Misdiagnosis as Herpes Simplex

Zoster: face and leg
• 110 patients with vesicular eruptions
  • Clinical diagnosis HSV (45) and zoster (65)
  • 65/65 clinical zoster diagnoses confirmed by VZ PCR
  • 36/45 clinical HSV diagnoses confirmed by HSV PCR
  • 9/45 (12%) clinical HSV diagnoses VZ PCR pos
  • Small eruptions periocular/nasolabial groove/thigh


Zoster: genital region
• 6210 patients with genital lesions; 2225 PCR pos
• 2185 (97%) HSV, 65 (2.9%) VZV
• 40/65 female, variety genital sites both sexes
• 5 clinically diagnosed VZV, 10 uncertain HSV/VZV
• VZV clinical diagnosis more common in children/elderly

**Varicella Zoster: Serology**

- IgM and IgG responses measurable within days of rash
- Many assay formats: IFA, EIA etc
- Adjunct to virus detection only
- Immunity of interest in at-risk exposures
  - Pregnant women
  - Nosocomial exposure
- Rapid formats often used eg latex agglutination

Varicella Zoster: Determination of Immunity

- Fluorescent anti-membrane antigen (FAMA) is serological gold standard
- EIA and LA correlate well and widely available
- EIA and LA validated against VZV CMI
- EIA (BioWhittaker)
  - 87% sensitive
  - 91% specific
  - 87% PPV
  - 91% NPV
  - Performance improved using “grey zone” of VI 0.9-1.2
- LA (Becton Dickenson)
  - 96% sensitive
  - 91% specific
  - 97% PPV
  - 90% NPV
  - Performance improved using 1:8 dilution rather than 1:2