Transplantation, Immunosuppression, Infection

Viruses in May 2010

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OUTLINE

- Transplant infections donor and recipient
  - Types
  - Pathogenesis
- Diagnosis of transplant infections
  - Laboratory diagnosis
  - Utility of diagnosis
- Emerging issues
  - New agents
  - Donor screening
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SOT – Recipients

- ~1800 Australians on wait list
  - 78% NSW/ACT and Victoria/Tasmania
  - Most kidney, liver
- Hospitalisation post transplant
  - Major reason infection
  - Resistant organism colonization
  - Continuing long term immunosuppression
SOT Infections Occurrence

- Epidemiologic exposure
  - Donor organ
  - Self
  - Hospital environment
  - Community

- Net state of immunosuppression
General Concepts - Recipients

- Increasingly potent immunosuppressive agents reduce rejection
- Increasing patient susceptibility to opportunistic infections and cancer
- Recognition of clinical syndromes (BK nephropathy, Adenovirus, Arenavirus)
- Routine antimicrobial prophylaxis
- Infections due to organisms with antimicrobial resistance
SOT Recipient Infection Types

- **BBV**
  - Herpesviruses (CMV, HSV, EBV, HHV6, HHV8)
  - HIV
  - HCV
  - HBV
  - HTLVI, II
  - Parvovirus
  - BK/JC

- **STI**
  - HPV

- **Emerging**
  - WNV
  - Lyssavirus
  - Arenavirus
  - Antibiotic resistant
  - Antiviral resistant CMV, HCV
The Timeline of Post-transplant Infections

Nosocomial Technical

Oppportunistic, Relapsed, Residual

From Common to rare

Transplantation 4 Weeks 6-12 Months Long-term

Donor-derived infection Nosocomial infection Period of most intensive immune suppression

Common Variables in Immune Suppression
- Rejection, anti rejection therapy, new agents
- Neutropena, lymphopaenia
- Viral coinfection (CMV, HCV, EBV)

[Fishman 2005]
## Estimated Number of Persons with Chronic Blood-borne Virus Infections 2000

<table>
<thead>
<tr>
<th>Region</th>
<th>Population millions</th>
<th>Chronic infections (millions)</th>
<th>HIV</th>
<th>HCV</th>
<th>HBV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Africa</td>
<td>749</td>
<td>22.7</td>
<td>22.5</td>
<td>59.3</td>
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<tr>
<td>Asia</td>
<td>3,585</td>
<td>7.3</td>
<td>107.5</td>
<td>286.8</td>
<td></td>
</tr>
<tr>
<td>Latin America</td>
<td>504</td>
<td>1.7</td>
<td>15.1</td>
<td>10.3</td>
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<tr>
<td>Europe</td>
<td>729</td>
<td>0.8</td>
<td>21.8</td>
<td>10.9</td>
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</tr>
<tr>
<td>Oceania</td>
<td>30</td>
<td>0.0</td>
<td>0.9</td>
<td>2.4</td>
<td></td>
</tr>
<tr>
<td>North America</td>
<td>305</td>
<td>0.9</td>
<td>9.1</td>
<td>1.9</td>
<td></td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>5,902</strong></td>
<td><strong>33.4</strong></td>
<td><strong>176.9</strong></td>
<td><strong>371.6</strong></td>
<td></td>
</tr>
</tbody>
</table>

[Margolis CDC]
Main Issues

- High-Risk donors
- Prospective/Retrospective testing
- Ideally universal prospective screening
- False Positives/Negatives
Transplant costs

- Transplant
  - $65,000-$75,000
  - $11,000 ongoing pa

- Post-transplant infection
  - Additional $5,000-$10,000 pa
  - Graft loss
  - Long-term damage
  - Cumulative (CMV → bacteria)
“…they belonged, at any rate, to the lowest and smallest but also to the most fruitful beings known…”

J. Henle 1833
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Pathogenesis
Mechanisms of chronic damage

- Up regulation growth factors
- Growth
  - MHC II upregulation
  - MHC II molecules ↑
  - Adhesion molecules ↑
- Chemokines
  - ↑ IL-2    ↑IL-2R
  - ↑ TNF
  - ↑ IL-6
  - ↑ PDGF + ↑ TGF β smooth
  - Muscle proliferation
CMV Pathogenesis

- Duration of treatment to reduce VL to O depends upon initial VL
- GCV
  - 10mg/kg/d IV 92% efficacy
  - 1g tds po 47% efficacy

[Emery 1999, 2000]
CMV antiviral resistance

- 37% of specimens contain antiviral resistant CMV sequences
- 14% have dual UL97+UL54 mutations
  - Confer multidrug resistance
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  ➢ Types
  ➢ Pathogenesis

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  ➢ Laboratory diagnosis
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• Emerging issues
  ➢ New agents
  ➢ Donor screening
Diagnosis

- Optimal methods vary with population

- Serology
  - ELISA IgG, IgM
  - IgG avidity

- Direct detection
  - direct immunofluorescence
  - NAT
  - Q-NAT
  - Quantitative antigenaemia
  - Viral culture
HV Infection  HV Disease

- Isolation of the virus from any site or serological evidence
- Primary or Secondary

- Invasive or symptomatic infection with histologic viral cytopathic effect
- Evidence of recent infection + clinical
Limitations of Serological Tests

- Longer window period than NAT
- Do not distinguish between HCV present or past infection
- HBV escape mutants are not detected
- Occult HCV and HBV infections are not detected
Summary of BBV testing at SEALS since October 2009

<table>
<thead>
<tr>
<th>Sample type</th>
<th>No. of donors tested by NAT</th>
<th>No. of organs retrieved</th>
</tr>
</thead>
<tbody>
<tr>
<td>Routine</td>
<td>26</td>
<td>89</td>
</tr>
<tr>
<td>Urgent (High-Risk donor)</td>
<td>10</td>
<td>21*</td>
</tr>
<tr>
<td>Total</td>
<td>36</td>
<td>110</td>
</tr>
</tbody>
</table>

*Two donors were rejected: one was HBV NAT Positive, and other was deemed not medically suitable*
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Current DDD Transmission Data

152 cases reported in 2009

# Reports to DTAC: 2006-2009*

<table>
<thead>
<tr>
<th>Malignancies</th>
<th># of Donor Reports</th>
<th># of Recipients w/ Confirmed Tx</th>
<th># of Attributable Recipient Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal Cell Carcinoma</td>
<td>60</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Lung – Adenocarcinoma</td>
<td>9</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Non-Hodgkins Lymphoma</td>
<td>6</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>Thyroid Cancer</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Prostate Cancer</td>
<td>5</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Melanoma</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Glioblastoma Multiforme</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hepatocellular Cancer</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Leukemia (AML, CLL)</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Ovarian Carcinoma</td>
<td>2</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Breast</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Colon Cancer</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neuroendocrine</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Pancreatic Adenocarcinoma</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Renal Papillary Adenocarcinoma</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Liposarcoma</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>11</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Totals</strong></td>
<td><strong>121</strong></td>
<td><strong>18</strong></td>
<td><strong>5</strong></td>
</tr>
</tbody>
</table>

*Other (Each are single cases with no transmission): Basaloid, Brain – Spindle Cell, Cholangiocarcinoma, Dermatofibrosarcoma Protuberans, GIST, Kaposi’s Scarcoma, Lung – Bronchoalveolar, Lung – Small Cell, Lymphoma, Myeloid Sarcoma, Urothelial Cell
Reports to DTAC: 2006-2009*

<table>
<thead>
<tr>
<th>Virus</th>
<th># of Donor Reports</th>
<th># of Recipients w/ Confirmed Tx</th>
<th># of Attributable Recipient Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCV*</td>
<td>26</td>
<td>8</td>
<td>1</td>
</tr>
<tr>
<td>HIV†</td>
<td>13</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>HBV</td>
<td>15</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>West Nile – UPDATE Outcome</td>
<td>12</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Parvovirus</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Influenza (2 Pandemic)</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>HTLV</td>
<td>2</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>LCMV</td>
<td>1</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>PIV-3</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Rabies</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Viral Encephalitis</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Viral Illness – Unidentified</td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
</tbody>
</table>

* All but 3 cases non-reproducible NAT results:
  - Donor not high risk, Sero -/NAT+ with 3 transmissions
  - Donor high risk, Sero -/NAT + with 4 transmissions (HIV/HCV) and 1 death
  - Donor HCV + vessel with 1 transmission

†All but 2 cases non-reproducible NAT results:
  - 1 Case with 4 transmissions of HCV/HIV and 1 death
  - 1 patient with HIV infection post-transplantation
Reports to DTAC: 2006-2009

<table>
<thead>
<tr>
<th>Bacteria</th>
<th># of Donor Reports</th>
<th># of Recipients w/ Confirmed Tx</th>
<th># of Attributable Recipient Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• <em>Acinetobacter</em> (2 cases)</td>
<td>25</td>
<td>9</td>
<td>6</td>
</tr>
<tr>
<td>• <em>Brucella</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• <em>Enterococcus</em> (3 Cases (1 VRE))</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• <em>E. coli</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• Gram Positive Bacteria</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• <em>Klebsiella</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• <em>Pseudomonas</em> (4 cases)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• <em>Serratia</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• <em>S. aureus</em> (2 cases, 1 MRSA)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• <em>Streptococcus</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• <em>Veillonella</em></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• 1 case of bacterial meningitis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>• 1 case of bacterial emboli</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>Ehrlichia</em></td>
<td>2</td>
<td>0</td>
<td>0</td>
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<tr>
<td><em>Legionella</em></td>
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<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Listeria</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Lyme Disease</em></td>
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<td>2</td>
<td>0</td>
</tr>
<tr>
<td><em>Nocardia</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Syphilis</em></td>
<td>7</td>
<td>2</td>
<td>0</td>
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<tr>
<td><em>Rocky Mountain Spotted Fever</em></td>
<td>1</td>
<td>0</td>
<td>0</td>
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</table>
# Other Infections

<table>
<thead>
<tr>
<th>Other Infections</th>
<th># of Donor Reports</th>
<th># of Recipients w/ Confirmed Tx</th>
<th># of Attributable Recipient Deaths</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mycobacterial Infections</strong></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>TB</td>
<td>24</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td><em>Mycobacteria avium</em>-complex</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><em>Mycobacteria kansasii</em></td>
<td>1</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td><strong>Fungal Infections</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coccidiodomycosis</td>
<td>6</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Histoplasmosis</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Zygomycetes</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td><em>Candida</em> spp</td>
<td>3</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Cryptococcus</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Aspergillus</td>
<td>2</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Mycotic Aneurysm</td>
<td>1</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td><strong>Parasitic Infections</strong></td>
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<td></td>
</tr>
<tr>
<td>Chagas</td>
<td>7</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Strongyloides</td>
<td>4</td>
<td>2</td>
<td>1</td>
</tr>
<tr>
<td>Schistosomiasis</td>
<td>3</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Babesiosis</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td>196 + 18$\S$</td>
<td>54</td>
<td>22</td>
</tr>
</tbody>
</table>

$\S$Expected Transmissions: 10 Toxoplasmosis, 7, EBV, 4 CMV. Data 1/1/06 – 10/31/09.
Human Cytomegalovirus

- Morbidity and mortality in immunocompromised
  - HIV-AIDS
  - transplant recipients
  - neonates

- SOT infections
  - lung > liver > heart > bowel > kidney

- Allograft injury best evidence linked
  - Heart CAD
  - Lung transplants Bronchiolitis obliterans
CMV risk factors

- CMV serostatus
  - D+/R-
  - D+/R+

- Coinfection
  - HHV6
  - HHV7

- Patient factors
  - HLA mismatch
  - T cell depletion
  - GVHD

- Therapy
  - High dose steroids
  - OKT3
  - Mycophenolate
  - CNI
CMV infection of kidney
CMV infection of lung
Quantitation of CMV

**Benefits**
- Surrogate measure of resistance
- Better correlation with disease
- Measure of viral load in blood vs other tissue
- Association with prognosis in some diseases
- Simplified sample

**Problems**
- Cost
- Lack of correlation with some disease
- Lower sensitivity than qualitative
- Availability
- Sample size for testing
QPCR use in transplant recipients

- Initiation of therapy in SCT
  - High risk allogeneic SCT 10,000 c/ml whole blood
  - Preemptive therapy with GCV 5mg/kg/dy
  - Dose escalation of GCV if no response of VL

- Initiation of therapy in renal transplants
  - Lower risk SOT 30,000 c/ml plasma

- Initiation of therapy in liver transplants
  - Moderate risk SOT 1,000 c/ml plasma, PPV 47%, NPV 83%
  - Moderate risk SOT 5,000 c/5x10⁶ cells, PPV 40%, NPV 90%

[Martin-Davila 2005; Rayes 2005; Verkruyse 2006]
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  ➢ New agents
  ➢ Donor screening
Emerging issues

- Zoonoses
  - WNV
  - Bat lyssavirus
  - Hendra/Menangle/Nipah

- Long-term immunosuppression + cancer
  - EBV
  - Endogenous retrovirus
  - HHV-8
New agents

- Imported infections
  - Dengue
  - New variant CJD
- Unexpected infections
  - Arenavirus
  - Seronegative HIV
  - Seronegative HCV
- Respiratory
  - Negative on routine testing
- Gastrointestinal
  - Negative on routine testing
OUTLINE

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Proposals

- Develop and institute pre-transplantation testing guidelines similar to those developed for US and expand those being developed for Australia
- Analysis of sensitivity, specificity and cost-effectiveness of different testing algorithms
- Develop and institute cadaver donor screening protocols
Sources of residual risk

- Infectious, window period donations
  - Time between infectivity and detection with screening tests may be different from time of exposure to an agent
- Viral variants
  - Strains, subtypes
  - Most not detected by current tests
- Infectious chronic antibody negative carriers
- Errors (testing or product release)
Comparative risk in medical procedures
Therapy

- **Antivirals**
  - ganciclovir (bone marrow toxicity)
  - foscarnet (renal toxicity)
  - adefovir
  - prophylaxis with ValGCV, ValACV, GCV, ValGCV

- Pre-emptive therapy
- Primary prophylaxis
- No effective vaccine
CMV antiviral resistance

- 47% CMV antiviral sensitive
- 15% CMV antiviral resistant - UL97
- 15% CMV antiviral resistant - UL97 + UL54
- 23% CMV PCR negative

- 38% of patients harbour antiviral resistant CMV
- 15% have dual UL97+UL54 mutations
Antivirals for resistant CMV

- Foscarnet
- Cidofovir
- Combination therapy
  - GCV 0.5 dose + PFA escalating to 125 mg/kg
  - GCV 0.5 dose + PFA 0.5 dose 90 mg/kg
  - GCV 1.0 dose + CMV IVIG
  - ?more toxic

[Mylonakis 2002; Mattes 2004]
Donor-Derived Infections

- Most are latent (CMV, TB, *T. gondii*)
- Rarely can be acute (Bacteremia/viremia at time of procurement i.e. West Nile, rabies, HIV, hepatitis, LCV)
- The majority of these are subclinical in healthy patients, but can be catastrophic when transplanted into an Immunosuppressed patient
- At present, routine evaluations of donors for infectious diseases relies upon serologic antibody testing, and thus sensitivity is not 100% for those that may not have had time to seroconvert
Donor Screening

- Epidemiologic history
- Serologic testing for VDRL, HIV, CMV, EBV, HSV, VZV, HBV (HBsAg, anti-HBsAg), and HCV
- Microbiologic testing of blood and urine
- Chest radiography
- Known infections (appropriate therapy?)
- Possible infections (e.g., encephalitis, sepsis)
- Special serologic testing, nucleic acid assays, or antigen detection based on epidemiologic factors and recent exposures (e.g., toxoplasma, West Nile virus, HIV, HCV)
NAT testing

- HCV
  - LOD 15 IU/ml (10 cp/ml)
- HIV-1
  - LOD 68 IU/ml (40 cp/ml)
- HBV
  - LOD 12 IU/ml (5 cp/ml)
- 3 Molecular tests run in parallel
- TAT 6-8 hrs
HIV markers during early infection

DT = 21.5 hrs

HIV RNA (plasma)

HIV p24 Ag

HIV Antibody

Theoretical Infectivity

HIV RNA

HIV p24 Ag

HIV Antibody

Day 0

Day 11

Day 16

Day 22

5 Days

6 Days