بسم الله الرحمن الرحيم
CMV in kidney Transplant recipient: A diagnostic and therapeutic Dilema

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Cytomegalovirus Virology

- CMV is a member of the genus herpesvirus and belong to the family herpesviridae.
- There are eight known HHV.
- Morphologically, HV are indistinguishable from one another.
## Human herpes virus and disease

<table>
<thead>
<tr>
<th>Virus</th>
<th>Disease and Possible Disease Associations</th>
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<tbody>
<tr>
<td>HSV 1</td>
<td>Herpes labialis</td>
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<tr>
<td>HSV 2</td>
<td>Herpes genitails</td>
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<tr>
<td>Viricella zoster virus</td>
<td>Chicken pox</td>
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<tr>
<td>Epstein-Barr virus</td>
<td>Infectious mononucleosis, Burkitt’s lymphoma, nasopharyngeal carcinoma, posttransplant lymphoproliferative disorder</td>
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<tr>
<td>Cytomegalovirus</td>
<td>Cytomegalovirus disease, salivary gland virus disease</td>
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<tr>
<td>HHV-6</td>
<td>Roseola (exanthem subitum), transplant encephalopathy, multiple sclerosis</td>
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<tr>
<td>HHV-7</td>
<td>Roseola</td>
</tr>
<tr>
<td>HHV-8</td>
<td>Kaposi’s sarcoma, body cavity-based lymphoma, sarcoidosis</td>
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Figure 1. Schematic of cytomegalovirus (CMV). The CMV virus shows a similar composition to all human herpes viruses.
CMV is a labila virus and readily inactivated by lipid solvents, PH below 5, heat (37°C for 1 hour or 56°C for 30 min.) and uv light for 5 min. it can survive on environmental surfaces for several hours.
White blood cells and CD 13 +ve cells in particular are the principal reservoir where CMV is harbored. It has been detected in most tissues in the body and may remain latent.
CMV antigens

• CMV replication produces immediate-early (IE), early, and late CMV antigens.
• IE antigens appear in the nucleus of CMV-infected cells 1 to 3 h after infection and remain present even in latent infection.
• Early antigens appear in the cytoplasm of approximately 3h after infection.
• Late antigens appear in the nucleus and cytoplasm within 6 to 24h after infection.
• IE and early antigens are virus-induced nonstructural proteins and appear before DNA synthesis.

• Late antigens are virally encoded structural proteins and appear after DNA synthesis.

• For early diagnosis ⇒ early antigens.

• For drug treatment (ganciclovic, foscarnet, and cidovir) monitoring ⇒ late antigen.
Worldwide seroprevalance of CMV is 30-100%.

- Found in body fluids

  Blood, Saliva, Urine, Breast milk.

- CMV is the most important viral infection affecting transplant recipients.

- In the Western countries, approximately 50% of patients waiting Tx have been infected in the past and have antibodies to CMV.
Types of CMV Infection

- **Primary infection**
  (asymptomatic to mononucleosis like syndrome in immune competent individuals)

- **Latent infection**
  (presence of viral genome in mononuclear leukocytes, endothelial cells, and organs in the absence of active replication of infectious virus)

- **Reactivation**

- **Reinfection**
  (new strain of CMV)
Patterns of Transmission of CMV in Solid Organ Transplant Recipients

**Primary Infection (D+/R-)**
- CMV-seronegative individual receives “cells” containing latent virus from a seropositive donor
- CMV is reactivated with clinical disease in 40% to 60% of D+/R- recipients in the absence of prophylaxis
- Asymptomatic viremia >50% without prophylaxis or seroconversion.
- Infection may also be acquired in the community or from blood products
Reactivation Infection (R+)

- CMV-seropositive recipient reactivates endogenous, latent virus posttransplantation.
- ~20% incidence of clinical disease without prophylaxis.
- High rate (40%-60%) of “asymptomatic” viremia.
Recurrent Infection After Therapy

- Usually inadequate course of antiviral therapy (dose or duration)
- Usually not a function of viral resistance, but resistance may occur—appropriate testing is available

Superinfection (D+/R+)

- Infection with multiple strains may occur
- May be of donor or recipient origin
Origin of CMV infection

- Transmission from staff or patients is unlikely.
- Whole-blood or leukocyte transfusion from CMV-seropositive donor can transmit the virus. The risk is greatest with fresh blood, and stored blood is potentially hazardous. Leukocyte-free blood appears to be safe.
- The main source of CMV infection is the transplanted kidney
- Reactivation of latent infection
- Restriction endonuclease analysis → to determine source of infection.
Risk Factors for CMV Infection

- Donor (D) and recipient (R) serologic status (prior immunity)—greatest in D+/R-
- Organ transplanted
- Nature and intensity of immune suppression—greatest with depleting antilymphocyte antibodies
- “Dose” of virus—usually in transplanted organ
- Rejection
- Other simultaneous infections (especially herpesvirus)
- Probably degree of MHC mismatch

 Relative Risk for Reactivation of Latent Viral Infection by Immunosuppressive Regimen

- Depleting anti–T-lymphocyte antisera (ATG, OKT3,++++ thymoglobulin), alloimmune effect (rejection)

- Cyclophosphamide, azathioprine, MMF ++

- Cyclosporine, prednisone, FK506, rapamycin ++

- Inflammation/fever (TNF via NF-κB) ++++

Some agents activate latent viral infection; others perpetuate infection by blocking development of immunity.

ATG, antithymocyte globulin; MMF, mycophenolate mofetil; TNF, tumor necrosis factor; NF-κB, nuclear factor kappa B.
CMV Infection vs Disease

- **CMV infection**: isolation of CMV or detection of viral proteins or nucleic acid from any body fluid or tissue specimen.

- **CMV disease**: combination of a clinical syndrome consistent with CMV and detection of CMV using an accepted assay or by biopsy of the affected organ.
Clinical Features

- A typical symptomatic CMV infection begins as a spiking or constant fever 4 to 10 weeks after Tx. Leukopenia, thrombocytopenia, and atypical lymphocytes often are present at this time.

- ↑ AST, ALT and development of respiratory symptoms (dyspnea with abnormal blood gases) begin several days after fever.
Less common features are arthralgia, overt hepatitis, splenomegaly, myalgia, abdominal pain and GI T ulceration and bleeding and encephalitis.

Skin vesicles are not feature of CMV infection

Renal dysfunction may be observed in this stage.
Fever may persist for a month or more

Most patients recover.

About 2% progress to develop disseminated fatal disease è pneumonitis, GIT bleeding.

Retinitis with perminant ↓ of visual acuity in 50% of cases is rare in kidney TX patients.
CMV Infection May influence the graft through

1. Fatal dissemination of CMV (1-2%) with severe depression of humoral-mediate and cell-mediated immunity, at P.M. no rejection.

2. In milder infections, humeral response are normal, graft function may be impaired by the fibrile illness.

3. Decreasing immunasuppression to overcome primary CMV infection may lead to acute rejection episeode

4. CMV glomerulonephritis
   Should be differentiated from acute vascular rejection

5. Acute rejection : Through increasing expression of MHC class II on CD₈ cells and class I and II on renal cells.

6. Immunosupressissive state with opportunistic infections due to ↓ production of 1L-1 and inversion of helper /suppressor (CD4/ CD8) T-lymphocyt ratio.
Cmv glomerulitis
Acute glomerulitis (humoral rejection)
Other pathogenic effects of CMV infection

- Atherosclerosis.
- Chronic rejection.
- Re-stenosis of coronary angioplasty.
- Transplant artery stenosis.
- HUS/TMA.
Chr rejection
Renal artery stenosis
HUS
Effect Of Donor CMV Status On CMV Infection In 306 Renal Allograft Recipients At Oxford

<table>
<thead>
<tr>
<th></th>
<th>Recipient CMV+ve</th>
<th>R. CMV-ve</th>
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<tbody>
<tr>
<td></td>
<td>D+ve</td>
<td>D-ve</td>
</tr>
<tr>
<td>Group total</td>
<td>84</td>
<td>98</td>
</tr>
<tr>
<td>No. infected</td>
<td>52 (62%)</td>
<td>52 (53%)</td>
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Reactivation | Primary infection
Does prevention of asymptomatic, noninvasive CMV infection have a long-term benefit?

Yes.

Does this outweigh the cost and toxicity of the drugs?

This is unknown.
Diagnosis

- It is important to distinguish between latency, Active infection (Viraemia, serologic response and/or excretion of the virus) and disease state.

- Early diagnosis of CMV disease is essential because early treatment is crucial.
Diagnostic Tests

- CMV can be isolated in human fibroblast culture (takes 5-28 days).

- PCR or direct CMV-antigen detection in PBL or FNAB pecimens (more rapid, detect infection 1 W before onset of disease) ⇒ allow preemptive treatment.
**CMV Antibody Assay**

- In primary and often in secondary infection, the appearance of CMV specific 1gM detected by gIM-ELISA usually occurs within 1-6 days of onset of symptoms (diagnostic), (transient).

- In the absence of 1gM, the appearance of CMV – specific 1gG in a primary infection or a greater than fourfold rise in titer is sought but may delay diagnosis for 1-2 weeks.
Biopsy specimens may be examined histologically for CMV inclusion antibodies or for the presence of CMV antigens. However, the sensitivity may be low.

The pp65 CMV antigenaemia test is now routinely used for the rapid diagnosis of CMV infection in immunocompromised patients.
Cmv inclusions
CMV pp65 antigenaemia test

Figure 4 CMV pp65 antigens detected in nuclei of peripheral blood neutrophils

(Virology Laboratory, New-Yale Haven Hospital)
Conventional cell culture is regarded as gold standard but requires up to 4 weeks for result.

More useful are rapid culture methods such as the DEAFF test which can provide a result in 24-48 hours.
Fig. 2. CMV centrifugation culture fixed and stained 16 hrs after inoculation showing viral proteins in nuclei of infected human fibroblast cells.
Surveillance cultures for CMV in blood or buffy coat have a useful overall predictive value (60%) of disease, but the presence of CMV in urine or throat washings is less useful.
In CMV-seronegative recipients of seropositive organ a program of weekly testing for the first 2-3 mon. after Tx using the antigenaemia assay or PCR has a positive predictive value of about 80% of detecting disease.
Prevention of CMV infection and disease

- Selection of seronegative blood and kidney donor.
- Live attenuated CMV vaccin in the pretransplant period. Despite early encouraging findings, currently there is no licened CMV vaccine.
- Choose the least aggressive imonunospression regimen that dose not compromise graft function and survival.
- Passive immunization with high titered CMV plasma or immune globulin or normal IVIG.
- Antiviral drugs
  - Acyclovir, Valacyclovir, Ganciclovir, Valganciclovir
Possible Prophylaxis for CMV

- **D+/R-**: IV ganciclovir in hospital, po valganciclovir x 3 months (6 months with anti-lymphocyte-antibody induction)
  - Repeat prophylaxis for ALS or antirejection therapy
- **D-/R-**: acyclovir or similar x 3 months (herpes simplex, VZV)
- **D-/R+**: IV ganciclovir in hospital, po valganciclovir x 3 months (6 months with anti-lymphocyte-antibody induction)
  - IV ganciclovir for ALS or graft rejection
  - May substitute routine quantitative monitoring after 3 months
- **Active disease**: treat until assay is negative, then 1 to 2 weeks beyond; prophylaxis with po x 3 months minimum

CMV, cytomegalovirus; IV, intravenous; ALS, antilymphocyte serum; VZV, varicella-zoster virus.
Valganciclovir for Prophylaxis in Solid Organ Transplantation

- Times to onset of CMV disease and to viremia were delayed with valganciclovir; rates of acute allograft rejection were generally lower with valganciclovir.
- Higher incidence of neutropenia with valganciclovir than with ganciclovir (8.2% vs 3.2%, respectively).
- “Once-daily oral valganciclovir was as clinically effective and well tolerated as oral ganciclovir tid for CMV prevention in high-risk SOT recipients.”

CMV, cytomegalovirus; SOT, solid organ transplant.
Treatment of CMV Disease

- Secondary CMV infections usually are mild, self-limiting ⇒ no specific treatment.
  Primary infections usually serious.
  ⇒ Proper treatment.
- If severe leukopenia or thrombocytopenia
  ⇒ ↓ immunosuppressive therapy temporary!
- Rejection episodes during CMV infection should not be treated with ATG or ALG.
- Ganciclovir is the cornerstone in treatment of CMV infection.
- Early treatment (preemptive) is mandatory to overcome serious diseases such as pneumonia.
Ganciclovir

- Ganciclovir effectively treat CMV-infected cells that contain a phosphokinase that is a product of the CMV ul 97 gene.

- This protein is capable of phosphorylating ganciclovir into an active moiety ageist CMV, EBV and HSV.

- By comparison, acyclovir has a very limited activity against CMV.
MECHANISM OF ACTION

Viral Protein Kinase (UL97)

GCV → GCV-MP → GCV-DP → GCV-TP

Cellular Enzymes

Inhibits Viral DNA Polymerase (UL54)

Extracellular → Intracellular
- Ganciclovir 5 mg/kg I.V. every 12h for 21d is effective for retinitis and sepsis, but it is less efficacious for more severe invasive CMV pneumonia such as pneumoral or GIT involvement.

- Hyperimmune CMV 1g addition may help in these settings.

- Cidofovir and foscarnet are used in ganciclovir resistance but are more toxic.

- It has a wide range of toxicity including neutropenia, thrombocytopenia and less often anaemia, also psycosis and nephrotoxicity.

- It is secreated mainly through kidney.
  - Dose modification with renal dysfunction.
• Ganciclovir is given orally in a dose of 1000 mg tid (low bioavailability 7%).

• Valganciclovir is a valyl-ester prodrug of oral ganciclovir, has a bioavailability of 70%, given in a dose of 450-900 mg daily, produces blood levels that are similar to I.V. administration of ganciclovir at 2.5 to 5 mg/kg.
CMV Resistance

• Mainly in AIDS and in lung transplant patients.

• Rare with kidney transplants. Mostly occurred on switching from I.V. to oral ganciclovir and use of MMF.

• Can be overcomed by ↓ immunosuppression, back to I.V. ganciclovir or using foscarnet.
Figure 2. Systemic exposure to ganciclovir (AUC24) after oral ganciclovir, intravenous ganciclovir and valganciclovir administration in liver transplant recipients. Data from [11,12]. The exposure of 450 mg valganciclovir (20.56 µg/h/ml) was noninferior to that of oral ganciclovir (20.15 mg/h/ml; 90% CI for relative bioavailability of 95 to 109%), while the exposure of 900 mg of valganciclovir (42.69 µg/h/ml) was nonsuperior to that of intravenous ganciclovir (47.61 µg/h/ml; 90% CI = 83 to 97%).
Virological efficacy of valganciclovir (VAL) versus ganciclovir (GAN) in post-transplant cytomegalovirus (CMV) prophylaxis
Tolerability of valganciclovir (VAL) versus ganciclovir (GAN) in solid organ transplant recipients

![Bar chart comparing side effects of VAL and GAN in transplant recipients]
## Dosage of oral valganciclovir in patients with impaired renal function

<table>
<thead>
<tr>
<th>CL\textsubscript{CR} (mL/min)</th>
<th>Induction dosage (mg)</th>
<th>Maintenance/prevention dosage (mg)</th>
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<tbody>
<tr>
<td>≥60</td>
<td>900 bid</td>
<td>900 od</td>
</tr>
<tr>
<td>40–59</td>
<td>450 bid</td>
<td>450 od</td>
</tr>
<tr>
<td>25–39</td>
<td>450 od</td>
<td>450 q2d</td>
</tr>
<tr>
<td>10–24</td>
<td>450 q2d</td>
<td>450 twice weekly</td>
</tr>
<tr>
<td>&lt;10</td>
<td>Not recommended</td>
<td>Not recommended</td>
</tr>
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\textit{bid} = twice daily; \textit{od} = once daily; \textit{q2d} = every 2 days.
WHAT DOES THE FUTURE HOLD

- Use of translational research to establish better predictive tools
  - Host response – CD8, CD4 responses to specific herpesvirus antigens
  - Viral factors – immune evasion gene expression
  - Understand the impact of herpesvirus interactions

- Novel targets – tailored drug therapy, selective immunosuppression, immunosuppression with co-existing antiviral activity

- Novel preventative strategies
  - Vaccine strategies- DNA vaccine, multi-epitope vaccines
  - Cell Mediated therapeutic modalities
THANK YOU

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