Clinical Applications of CMV Viral Load Assays in Transplantation

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Potential Impact of Reclassification of CMV VL assays: *Assumptions & Concerns*

- Reduced barrier to FDA-approved assays
  - more commercial assays available (particularly if LDT's are limited)
  - greater variability [Preiksaitis *Clin Infect Dis* 2016]
- Potential for increased negative impact of CMV transplantation (unless appropriate special controls in place)
- Current situation:
  - multiple LDT's in widespread use
  - variably evaluated

CMV in Transplantation: BACKGROUND

- CMV has a major negative impact on transplantation
  - Direct morbidity & mortality (closely linked to viral load)
    - HCT: end-organ disease (GI, hepatitis, pneumonia, retinitis, etc.)
    - SOT: CMV syndrome, CMV end-organ disease (GI, hepatitis, pneumonia, retinitis, etc.)
  - Cellular biological effects (less well-established link to viral load)
- Risk factors for CMV disease generally well-defined
  - HCT: R+ > D+R-, stem cell source (haplo, cord), donor type (mismatched, unrelated > other), intensity of immunosuppression
  - SOT: donor/recipient CMV serostatus pre-transplant (D+R- > R+ > D-R-), type of organ transplant (lung/heart > other), intensity of immunosuppression (lymphocyte-depletion therapy)

CMV VL Assays in Clinical Transplantation

- Widely used
- Incorporated into major transplant guidelines [KDIGO, AST ID COP, CMV International Consensus]
- Indications are expanding (site-specific testing: BAL, CSF, biopsies, etc.)
- A few built-in safeguards:
  - Used in conjunction with other clinical/lab data
  - Serial testing (trends)

Principles Underlying Use of CMV VL Assays in Transplantation

- Absolute viral load in blood predicts disease risk (static)
- Rate of increase in blood viral load predicts disease risk (dynamic/kinetic)
- Threshold concept of CMV pathogenesis
  [Griffiths & Emery Clinical Virology: Cytomegalovirus, 2002]

Reviewed in Razonable & Hayden *Clin Microbiol Rev* 2013

Major Indications for CMV Viral Load Testing in Transplantation

1. Diagnosis of CMV syndrome (unique to SOTx)
2. Adjunct to diagnosis of end-organ disease (de-emphasized in recent guidelines [Ljungman *Clin Infect Dis* 2016])
3. Marker to guide preemptive therapy (PET)
4. Monitoring response to therapy

What aspects of CMV VL assays matter to clinicians?

- Sensitivity/Lower limit of detection
- Ability to assess a “true change” in viral load across a broad range of viral loads
- Clinically significant VL threshold

Diagnosis of CMV Syndrome

Current definitions [Ljungman *Clin Infect Dis* 2016]

- Proven—NOT DEFINED (impossible to exclude other causes)
- Possible: NOT DEFINED
- Probable: CMV in blood + clinical and/or lab abnormalities
- Issues/Challenges:
  - no specific viral load threshold for “clinical significance” (probably varies by specific patient population)
  - significant variability in sensitivity among assays
  - do all assays measure the same thing (intact virions, “free” DNA fragments, etc.)
  - multiple viral etiologies for “CMV syndrome”

Adjunct to Diagnosis of End-organ CMV Disease

- Detection of CMV in blood is no longer part of definition for end organ disease of any type [Ljungman CID 2016]
- Definition:
  - Proven/Probable: clinical symptoms AND demonstration of CMV in biopsy specimen (viral culture, histopathology)
  - Possible category: qPCR on biopsy (and other clinical criteria)

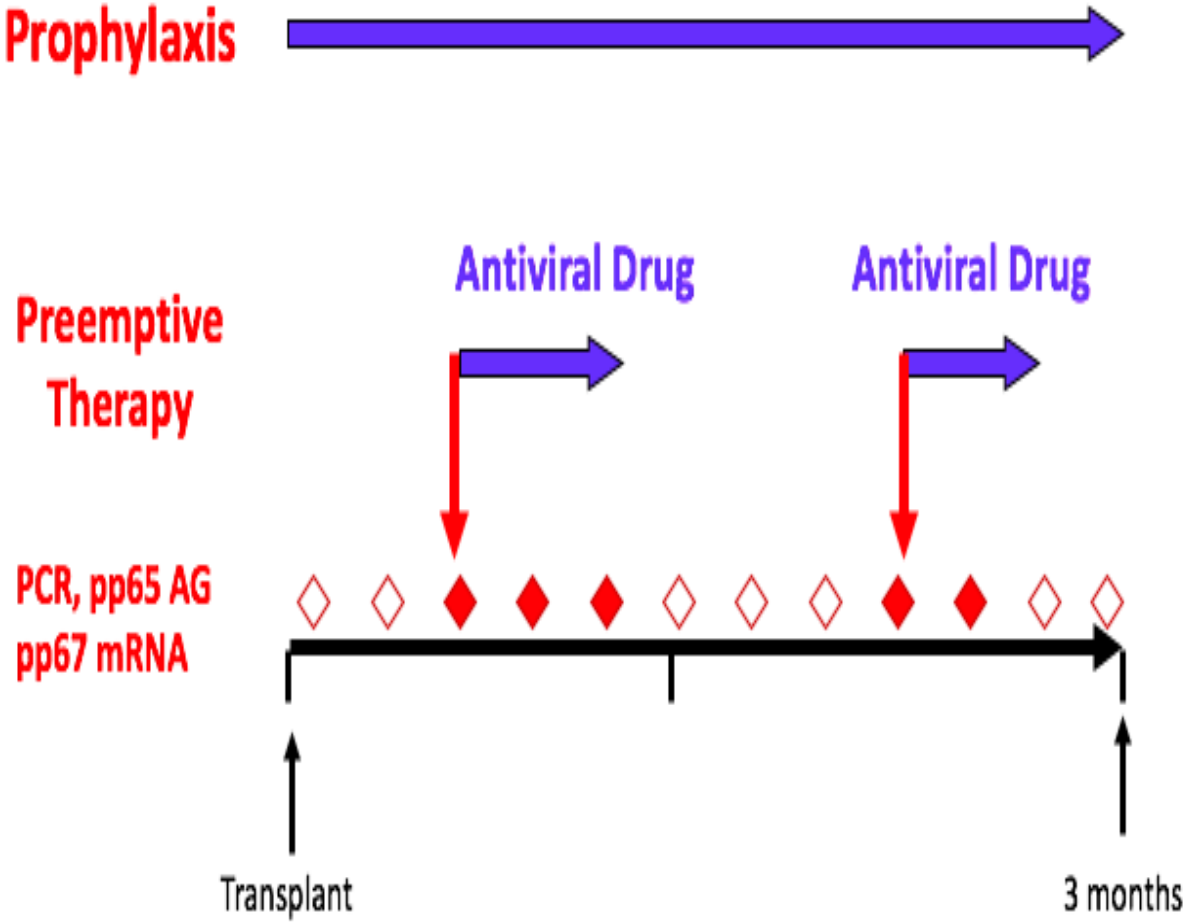
Adjunct to Diagnosis of End-organ CMV Disease (2)

Limitations/issues:

- Biologic:
  - "compartmentalization"/local reactivation not reflected in blood VL (GI disease, retinitis, CNS disease, CMV pneumonia in lung transplant)
  - lack of specific threshold with 100% sensitivity or specificity for all CMV disease in all populations
- Non-Biologic (assay-related--Cook)
  - Inter-assay variability
  - Specimen type (WB vs Plasma vs PBMC)
  - Inability to directly compare VL across labs/assays:
    - Individual patient care (transplant center vs local lab)
    - Interpretation of data across centers

Marker to guide Preemptive Therapy (PET)

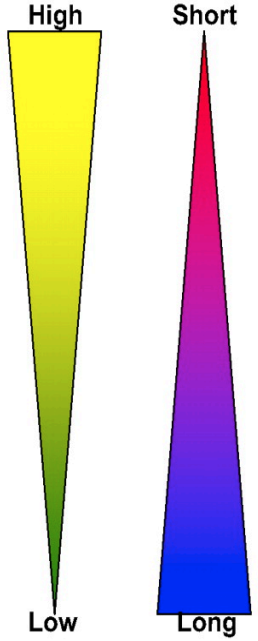
- 2 major strategies for CMV prevention:
  - Prophylaxis
  - PET
- Both strategies are recommended for most transplant settings



Importance of Specific Assay Characteristics for Guiding PET

Initiation of preemptive therapy in HCT recipients based on:

- Absolute VL thresholds based on patient risk strata (sensitivity)
- Viral kinetics

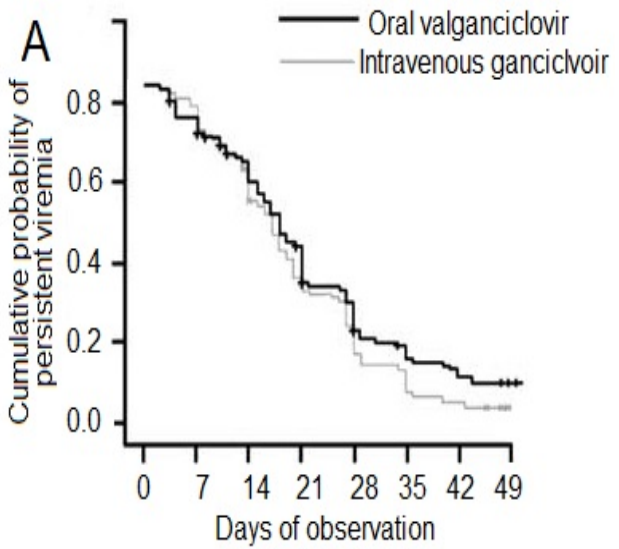


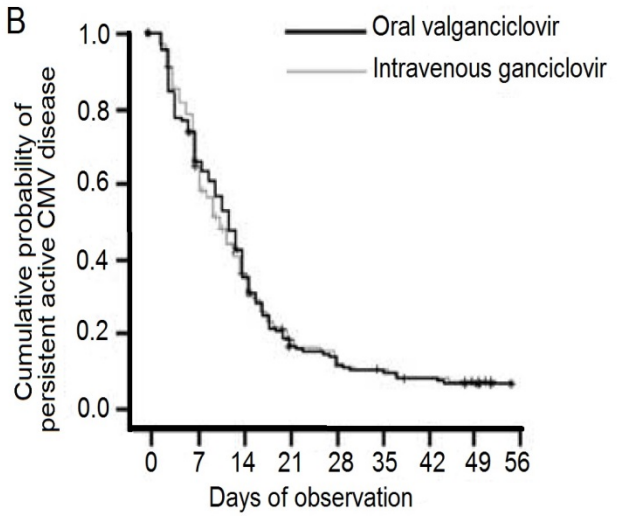
Immuno-suppression	CMV doubling time	Risk Groups	CMV Plasma DNA Level to Start PET at FHCRC*	CMV Whole Blood DNA Level to Start PET at Karolinska Institute**
High	Short			
		Cord blood	Any level	1000 copies
		Allograft - High-dose steroids† - T cell depletion - Anti-T cell antibodies - CD34 selection	> 100 copies/mL	1000 copies
		Allograft - Low dose steroids - No T cell depletion or anti T cell antibodies	> 500 copies/mL > or 5-fold ⇑ †	1000 copies
		Allograft - after day 100	> 1000 copies/mL > or 5-fold ⇑ †	1000 copies if GVHD Other individual assessment based on ⇑
Low	Long			

Boeckh & Ljungman *Blood* 2009

Monitoring response to therapy

- Expected response to therapy [Asberg *Am J Transplant* 2007]
  - Clinical—improvement/resolution of symptoms by 2 weeks
  - Virologic—reduction in VL within 2 weeks
    - Resistance predicted by virologic failure (trigger for resistance testing)
- Viremia at end of treatment is independently associated with risk for recurrence [Razonable *Clin Microbiol Rev* 2013]
  - Differences in assay sensitivity➔ impact therapy duration [Lisboa *Transplantation* 2011]





cont. Monitoring response to therapy

- Ganciclovir resistance is an important concern
- Alternatives to ganciclovir are highly toxic
- Limitations of current assays (direct detection of genotypic resistance):
  - Slow TAT
  - Variable interpretation/reporting [Limaye ICAAC 2012]
  - Relatively expensive
- Accurate changes in VL➔ important to guide:
  - Need for CMV resistance testing
  - Risk/benefit of empiric change to more toxic therapy [Avery *Transplantation* 2016]

Emerging uses of CMV VL assays: Blood & Beyond

- Site-specific testing:
  - CSF—CNS disease (encephalitis, ventriculitis)
  - BAL—pneumonia
  - GI or other biopsy specimens
- Yet an additional variable & layer of complexity…

CMV VL Assays in Transplantation: Current Status

- Major issues with across lab assay comparisons:
  - Generally known among transplant physicians
  - Complicates post-transplant care (decentralized care)
  - Approach: try to have all assays performed at same lab (difficult)
- Clinicians have little input into laboratory assays
  - "quality" of assay is presumed
  - little or no data to end-users:
    - assay performance
    - clinical correlation

Potential outcomes of reclassification of CMV VL Assays—The Good

- barriers to commercialization decreased→ more available assays→ less expensive?
- greater availability for local/on-site testing→ shorter TAT
- might facilitate greater use of PET (access to frequent testing with short TAT required)

Potential outcomes of reclassification of CMV VL Assays: Concerns

- more assays➔ greater variability ➔ greater difficulty in interpretation
- "lower quality" assays➔ negative clinical impact
  - inadequate/variable sensitivity:
    - breakthrough disease when using PET
    - inadequate duration of therapy (higher risk of recurrence)
  - inadequate quantitation:
    - over or under-diagnosis of resistance
    - inappropriate duration of antiviral therapy