Use of Viral Load Testing in Managing CMV Infections in SOTR

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Disclosures

Scientific Advisory Boards:
   Roche Molecular, Quidel, Cepheid, Mesa Biotechnology, bioMeriuxex

Clinical Trials:
   T2 Biosystems, Hologic
Overview

- Update on CMV viral load testing performance
- Clinical uses of viral load testing
- Role of cell mediated immunity tests
Quantitative CMV Testing

- Two commercial assays that are FDA approved
  - Cobas AmpliPrep/Cobas TaqMan Test (Roche)
  - artus CMV RGD MDx Test (Qiagen)
- Variety of CE Marked and ASR reagents
The WHO CMV International Standard

- Biological standard, cultured virus (Merlin Strain) in universal buffer
- Worldwide testing to assign a consensus value in international units
- Has the WHO International Standard Improved Agreement?
Multi-Center CMV Study
Post WHO International Standard

Clinical samples sorted by increasing geometric mean (GM) of results for each sample

Preiksaitis JK et al. CID 2016: DOI:10.1093/cid/ciw370
Intra-laboratory and Inter-laboratory variability

Preiksaitis JK et al. CID 2016: DOI: 10.1093/cid/ciw370
Amplicon Size

CMV DNA in plasma is fragmented

Preiksaitis JK et al. CID 2016: DOI:10.1093/cid/ciw370
Analysis of CAP Proficiency Data

• Surveys from 2013
  ▪ VLS and VLS2 surveys

• 504 laboratories reported CMV results in $\log_{10}$ copies/ml

• 157 laboratories reported CMV results in $\log_{10}$ IU/ml
Box plot of CMV viral load (IU/mL vs copies/mL)

RT Hayden, Y Sun, L Tang, GW Procop, DR Hillyard, BA Pinsky, SA Young, AM Caliendo. Manuscript in preparation
Box Plot of CMV viral load by assay in IU/mL

RT Hayden, Y Sun, L Tang, GW Procop, DR Hillyard, BA Pinsky, SA Young, AM Caliendo. Manuscript in preparation
Unnecessary variability: no standardized method to quantify secondary standards

Hayden et al. JCM 2015; 53:1500.
FIG 3 Regression analysis of real-time PCR measures compared against nominal values stratified by assay.

FIG 4 Regression analysis of real-time PCR measures compared against the average ddPCR measure stratified by assay.
Impact of Specimen Type

• Whole blood versus plasma

• Viral load values in most patients are about $1 \log_{10}$ higher in whole blood vs plasma
  - Some patients the difference was as great as $2 \log_{10}$
  - Occasionally plasma viral load was found to be higher than whole blood viral load

Reducing Variability Between Assays

- Report values in international units
  - Agreement of viral load values is improving with WHO international standard
  - Variability still exists
- Using commercial assays
  - The more complete the better (extraction, standards)
- Quantifying secondary standards with digital PCR
- Consistent specimen type
Clinical Uses of CMV Viral Load Testing

• Decisions regarding initiating preemptive therapy

• Hybrid approach
  ▪ Monitoring after a period of prophylaxis
  ▪ Increased use of prophylaxis has led to increased cases of late disease
  ▪ Difficult logistics

• Diagnosis of CMV disease
  ▪ What viral load correlates with disease
Clinical Uses of CMV Viral Load Testing

• Monitoring response to therapy
  ▪ Baseline viral load the day antiviral therapy is initiated
  ▪ Test weekly
  ▪ Treat at least two weeks, until 1 or 2 tests are below the limit of detection
  ▪ Can use secondary prophylaxis (high risk; D+/R-, high initial viral load, high net state of immunosuppression, lung transplant, GI tissue invasive disease)

• Assessing treatment failure, resistance
What viral load correlates with disease?
ROC Curve CMV VL: Liver Transplant

- PCR > 400 copies/ml - disease
  Sensi 100%, speci 47%, PPV 34%, NPV 100%

- PCR > 2,000 copies/ml - disease
  Sensi 91%, spec 75%, PPV 50%, NPV 99.6%

- PCR > 5,000 copies/ml - disease
  Sensi 86%, spec 87%, PPV 64%, NPV 96%

[A. Humar et al., Transplantation 1999, 68:1305-1311]
Prospective cohort study

- Derivation and validation cohort
- CMV sero-positive SOTR
- Excluded induction therapy with anti-lymphocyte antibody
- Testing every 2 weeks for 100 days, then every 4 weeks until 180 days
- Viral load test, Roche Light Cycler test (IU/ml)
  - Magna Pure Compact extraction
### Table 2
Demographic characteristics of the patients.

<table>
<thead>
<tr>
<th></th>
<th>Derivation cohort</th>
<th>Validation cohort</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients included, no. (%)</td>
<td>141</td>
<td>252</td>
</tr>
<tr>
<td>Age, median (range), year</td>
<td>53 (19–73)</td>
<td>55 (19–75)</td>
</tr>
<tr>
<td>Sex, men, no. (%)</td>
<td>92 (65.2)</td>
<td>164 (65.1)</td>
</tr>
<tr>
<td>Solid organ transplant, no. (%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kidney</td>
<td>49 (34.8)</td>
<td>87 (34.5)</td>
</tr>
<tr>
<td>Liver</td>
<td>13 (9.2)</td>
<td>30 (11.9)</td>
</tr>
<tr>
<td>Heart</td>
<td>29 (20.6)</td>
<td>48 (19.1)</td>
</tr>
<tr>
<td>Episodes included, no. (%)</td>
<td>84</td>
<td>119</td>
</tr>
<tr>
<td>Preemptive therapy, no. (%)</td>
<td>29 (34.5)</td>
<td>60 (50.4)</td>
</tr>
<tr>
<td>CMV disease</td>
<td>9 (10.7)</td>
<td>9 (7.6)</td>
</tr>
</tbody>
</table>

### Table 3
Real Time-PCR CMV performed.

<table>
<thead>
<tr>
<th>RT-PCR range (IU/ml)</th>
<th>Derivation cohort no. of samples (%)</th>
<th>Validation cohort no. of samples (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>267 (27.2)</td>
<td>288 (14.2)</td>
</tr>
<tr>
<td>≤1000</td>
<td>112 (11.4)</td>
<td>48 (2.4)</td>
</tr>
<tr>
<td>1001–3000</td>
<td>114 (11.6)</td>
<td>166 (8.2)</td>
</tr>
<tr>
<td>3000–10,000</td>
<td>26 (2.6)</td>
<td>59 (2.9)</td>
</tr>
<tr>
<td>10,001–200,000</td>
<td>13 (1.3)</td>
<td>15 (0.7)</td>
</tr>
<tr>
<td>≥200,001</td>
<td>2 (0.2)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Negative</td>
<td>715 (72.8)</td>
<td>1734 (85.8)</td>
</tr>
<tr>
<td>Total</td>
<td>982 (100)</td>
<td>2022 (100)</td>
</tr>
</tbody>
</table>
Validation Cohort

<table>
<thead>
<tr>
<th>Threshold (IU/ml)</th>
<th>Specificity % (IC95%)</th>
<th>Sensitivity % (IC95%)</th>
<th>PPV % (IC95%)</th>
<th>NPV % (IC95%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6319</td>
<td>93.0 (89.2-95.5)</td>
<td>55.6 (26.7-81.1)</td>
<td>21.7 (9.7-41.9)</td>
<td>98.4 (95.9-99.4)</td>
</tr>
<tr>
<td>4220</td>
<td>89.9 (85.6-93.0)</td>
<td>77.8 (45.3-93.7)</td>
<td>21.2 (10.7-37.8)</td>
<td>99.1 (96.9-99.8)</td>
</tr>
<tr>
<td>3983</td>
<td>89.9 (85.6-93.0)</td>
<td>88.9 (56.5-98.0)</td>
<td>23.5 (12.4-40.0)</td>
<td>99.6 (97.6-99.9)</td>
</tr>
<tr>
<td>3194</td>
<td>100 (70.1-100)</td>
<td>88.4 (83.9-91.7)</td>
<td>23.1 (12.6-38.3)</td>
<td>100 (98.9-100)</td>
</tr>
</tbody>
</table>

• Median viral load with disease: 5550 IU/ml
• 2 patients had disease with a viral load below the cutoff
  ▪ 3324 IU/ml: gastritis in renal transplant
  ▪ 1930 IU/ml: gastritis in liver transplant
• 1 of 393 patients developed disease without viremia
  ▪ Gastritis in liver transplant
Issues to Consider

- Plasma based assay
  - Results with whole blood will be higher in most cases, on average $1\log_{10}$
- This cut-off applies to low risk SOTR
- Cut-off is likely specific to the assay, including the extraction method.
  - IU/ml helps but does not completely eliminate variability
What is a significant change in a viral load value?

Are those viral load values really any different?

Need to know the reproducibility of the assay.
Changes in viral load of 3-5 fold

Cobb, Lee, Boisvert, Duncan, Baum, Do, Caliendo, Asberg, Yao, Razonable unpublished data
Current state: CMV genotypic testing

- Sequencing of UL97 and UL54 genes directly from plasma sample
  - Need a viral load of at least 1000 IU/ml
  - Next generation sequencing
- Need to know the genetics of resistance
- Results are available in ~1 week
- Identifying at risk patients
  - Persistent viral load, increasing VL
  - Prolonged drug exposure (median 5-6 mos)
  - D+R- , lung transplant recipients

Mutations causing low and high level resistance

GCV = ganciclovir; FOS = foscarinet; CDV = cidofovir
[1] Symptomatic disease or viral load not improving
[2] Full dose GCV = 5 mg/kg bid i.v.
   High dose GCV = 10 mg/kg bid i.v.
   (adjust doses for renal function)

Tests of Cell Mediated Immunity

- Interferon-γ release assays
  - QuantiFERON-CMV
    - Measures release of interferon-γ by CD8+ T-cells after stimulation with CMV specific antigens
  - ELISpot
    - Detects release of interferon-γ by CD4+ and CD8+ T-cells in CMV antigen-stimulated PBMCs
ELISPOT assisted analysis

- Microtiter plate
- T cells + peptide
- Anti-IFN-γ antibody
- HRP-labelled antibody

1. Wash off cells
2. Add HRP substrate
3. Harvest plasma and test IFN-γ levels using ELISA
4. Read absorbance and analyse results using computer-based software

QuantiFERON®-CMV assisted analysis

- No peptides (control)
- HCMV peptides
- Mitogen control

Incubate tubes at 37°C for 16-24 h
Pre-transplant risk stratification in sero-positive patients

• Prospective study, lung and renal transplants
  ▪ 11/11 R- where non-reactive (QuantiFERON)
  ▪ 30/44 R+ reactive, 14/44 R+ non-reactive

• R+ nonreactive 7/14 (50%) developed post-tx CMV replication versus 4/30 (13%) in R+ reactive

• R+ lacking CMI have increased risk of reactivation
  ▪ Clinical trial going on to determine if these patients should be managed as high risk

Cartisán S et al. AJT 2013;13:738
Late onset CMV, after discontinuing prophylaxis

- High risk patients enrolled
  - D+/R-, R+ with thymoglobulin induction therapy, lung transplant (except D-/R-)
  - QuantiFERON CMV at baseline, 1, 2 and 3 months post transplant
  - 108 patients, D+/R+ = 39, D-/R+ = 34, D+/R- = 35
  - 18 developed symptomatic CMV disease
  - Patients received prophylactic therapy

Kumar D AJT 2009;9:1214
Monitoring CMI may be useful in predicting late onset CMV disease
Assessment of Cytomegalovirus-Specific Cell-Mediated Immunity for the Prediction of Cytomegalovirus Disease in High-Risk Solid-Organ Transplant Recipients: A Multicenter Cohort Study

- Adult SOTR, D+/R- patients who received prophylaxis
- QuantiFERON CMV testing end of prophylaxis, 1 and 2 months

<table>
<thead>
<tr>
<th>QT Result</th>
<th>No. (%)</th>
<th>CMV Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>31 (25%)</td>
<td>6.4%</td>
</tr>
<tr>
<td>Negative</td>
<td>81 (65%)</td>
<td>22%</td>
</tr>
<tr>
<td>Indeterminate</td>
<td>12 (10%)</td>
<td>58%</td>
</tr>
</tbody>
</table>
Figure 2. Kaplan-Meier curves of the incidence of cytomegalovirus (CMV) disease according to the result of the Quantiferon-CMV assay. A, Positive vs negative vs indeterminate result of the assay (log-rank test, \(P<.001\)). B, Positive vs nonreactive result of the assay (log-rank test, \(P=.024\)). Abbreviation: CMV, cytomegalovirus.
Spontaneous Clearance of Asymptomatic CMV Viremia

• Prospective study SOTR, developed asymptomatic low level viremia, not requiring antiviral therapy

• CMI measured shortly after viremia and longitudinally (QT assay)

• 37 patients
  ▪ Median viral load 1140 copies/ml
  ▪ Spontaneous clearance:
    • +QT 24/26 (92%); -QT 5/11 (46%)

Viral load, CMV-specific T-cell immune response and cytomegalovirus disease in solid organ transplant recipients at higher risk for cytomegalovirus infection during preemptive therapy

Cecilia Martín-Gandul,¹,²* Pilar Pérez-Romero,¹,²* Pilar Blanco-Lobo,¹,² Omar J. Benmarzouk-Hidalgo,¹,² Magdalena Sánchez,¹,² Miguel A. Gentil,²,³ Carmen Bernal,⁴ José M. Sobrino,⁵ María J. Rodríguez-Hernández,¹,² Elisa Cordero¹,² and The Spanish Network for Research in Infectious Diseases (REIPI)

D+/R- SOTR

Summary

• Agreement of viral load values is improving with WHO international standard
  ▪ Variability still exists
  ▪ Reduce with commercial assays, standard specimen type, quantifying standards with digital PCR

• Some data on cut-off for preemptive therapy in IU/ml, still assay dependent (~4000 IU/ml)
Summary

- CMI (QuantiFERON CMV) testing
- Accumulating data on utility when used with viral load testing
  - Pre-transplant risk stratification in sero-positive pts
  - Assessing risk of late-onset CMV disease after antiviral prophylaxis
  - Predicting patients likely to spontaneously clear asymptomatic viremia (DNAemia)