Cytomegalovirus antiviral resistance testing
A New Assay for Detection of Mutations Associated with Antiviral Resistance in CMV

Presenter:
Matthew J. Binnicker, Ph.D., D(ABMM)
Director of Clinical Virology
Department of Laboratory Medicine and Pathology
at Mayo Clinic, Rochester, Minnesota
Disclosures

• None

Cytomegalovirus (CMV) in the transplant population

• High seroprevalence, with possibility for acute and latent disease
• One of the most common complications affecting transplant recipients
• Presentation depends on a variety of factors:
  • Type of transplant (stem cell vs. solid organ)
  • Serostatus of recipient and donor (e.g., D+/R- vs. D+/R+)
  • Degree of immunosuppression
Potential clinical manifestations in the transplant population

• “CMV syndrome”
  • Fever of unknown origin >48 hours
  • Malaise
  • Decreased neutrophil count
• Pneumonitis
• Hepatitis
• Gastrointestinal disease (e.g., esophagitis, colitis)
• Central nervous system disease

Diagnosis of CMV

• Molecular detection of CMV nucleic acid in clinical specimens
• Increasing viral load ($\geq 0.5 \log$) in serially-collected plasma samples
• Histopathology of infected tissue (i.e., Owl's-eye inclusion)

CMV disease: Presence of CMV in clinical specimen(s) accompanied by consistent clinical manifestations
Treatment of CMV in transplant recipients

- Reduction of immunosuppression
- Antiviral therapy (ganciclovir, valganciclovir, foscarnet, cidofovir)
- Resistance to antiviral therapy may occur:
  - ~1-5% of transplant recipients
  - Results from mutation(s) in 2 primary CMV genes:
    - UL97 – can confer resistance to ganciclovir
    - UL54 – less common; can confer cross-resistance (ganciclovir/cidofovir, foscarnet)

When to suspect CMV antiviral resistance?

- Rising or persistently elevated viral loads despite appropriate treatment for >2 weeks
Options for CMV antiviral resistance testing

• Phenotypic testing
  • Requires viral culture in presence and absence of antiviral drug
  • Takes weeks to months; therefore, no longer used

• Sanger sequencing
  • Conventional sequencing of regions of DNA (i.e., UL97)
  • Can only sequence short pieces of DNA (300-900 bp)
  • Sequence quality drops after ~700 bp
  • May not be able to accurately differentiate mixed populations of sequence

Future option for CMV antiviral resistance testing

• Next-generation sequencing (NGS) assay for detection of mutations in UL97/UL54 associated with resistance
CMV next-generation sequencing assay

• Implemented at Mayo Clinic Laboratories on May 2, 2019
• Requires a plasma sample with a CMV viral load of ≥500 international units (IU)/mL (Sanger typically requires viral load ≥1,000 IU/mL)
• Demonstrated 97.7% (43/44) overall agreement with Sanger sequencing for resistance-level determination
• Can identify resistance-associated mutations at a prevalence threshold of ≥15% (vs. ≥20% by Sanger)

Summary

• CMV is an important cause of disease in transplant patients
• Resistance to antivirals can occur and results from specific mutations in:
  • UL97 – resistance to ganciclovir
  • UL54 – resistance to multiple antivirals
• Next-generation sequencing offers a novel approach to detect mutations associated with antiviral resistance
References


Thank you.