Title Sequencing drug-resistant cytomegalovirus in paediatric patients: towards personalised medicine

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Summary (117 words)

Cytomegalovirus is an ubiquitous herpesvirus that causes silent-to-mild infections in healthy individuals, and potentially fatal infections in the immunocompromised, especially paediatric patients. CMV reactivation during periods of intense immune suppression is associated with significant economic costs and poor patient outcomes. With a limited range of drugs licensed to treat CMV reactivation, managing antiviral resistance is vital. In research settings, high-throughput sequencing is superseding PCR-based monitoring of resistance mutations, revealing a more complicated – and more informative – picture of emerging mutation profiles in clinical samples. In the next decade, it is foreseeable that CMV whole genome sequencing for management of antiviral drug resistance will become as important for personalised patient care as qPCR monitoring of virus loads is today.
Cytomegalovirus (CMV) is a common human pathogen causing life-long infection. In immunocompetent individuals, this double stranded beta herpesvirus causes a silent or mild primary infection, occasionally causing an infectious mononucleosis-like illness. In individuals with iatrogenic, acquired or inborn immunodeficiency, CMV has the capacity to cause a range of diseases. These include pneumonitis, colitis, CNS disease, and ocular manifestations such as retinitis and uveitis; the more severe forms of CMV disease can be fatal.

Paediatric patients may be at risk of severe CMV infections for a number of reasons. There is data to suggest that while the paediatric CD8+ T cell response to primary CMV infection is similar to that of adults [1], the CD4+ response is not as effective as the adult response [2]. Primary CMV infections in infants leads to prolonged shedding of the virus in urine and saliva which is not seen in adults [3], with the result that infants may present a significant infection risk to their siblings and other children they interact with closely, as well as CMV-negative adults.

There are four major categories of paediatric patient in whom serious CMV disease is commonly reported. Congenital CMV infection occurs in ~0.5% of live births, with the greatest risk found in mothers who are infected with CMV for the first time during pregnancy. Children and adults with HIV are at significant risk of CMV disease due to poor T cell control of this virus. Reduced T cell function contributes to the increased risk of CMV disease in organ transplant recipients, as intense immune suppression is required to prevent graft rejection. Finally, inborn errors of immunity and conditions leading to poor thymus development also put children at risk of serious CMV infections and disease. CMV is therefore a significant pathogen in many areas of paediatric medicine.

The burden of drug resistance in cytomegalovirus disease

There are five drugs available for treatment of CMV (ganciclovir, cidofovir, foscarnet, maribavir and letermovir), and a number of other drugs used off-label to treat CMV disease, such as leflunomide and artemesunate. These drugs may be given
prophylactically to prevent CMV reactivation, or pre-emptively to treat emerging CMV
disease, but either approach requires weeks to months of antiviral therapy. These drugs
have significant side-effect profiles of their own, creating a risk that antiviral treatment
may need to be reduced or stopped. Some CMV antivirals poorly penetrate certain
tissue compartments. Both factors increase the likelihood of paediatric patients being
exposed to sub-therapeutic drug doses, a risk factor for emergence of antiviral
resistance.

The economic burden of CMV reactivation in the setting of paediatric transplantation,
especially haemopoetic stem cell transplant (HSCT), has been calculated as up to
£22,500 (~$34,000) per patient, as well as CMV disease increasing the risk of graft
rejection in solid organ transplant recipients [4, 5]. A recent study found that pre-emptive
antiviral treatment led to 14.5% of CMV-positive haplo-HSCT recipients developing
antiviral resistance mutations [5]. Ensuring timely and effective delivery of antivirals and
preventing resistance is also important for long-term patient outcomes in congenitally
infected neonates. Antiviral therapy can reduce or prevent many of the long-term
sequelae of congenital CMV infection, including sensorineural hearing loss [6]. Given
the small pool of antiviral options currently available to clinicians, there are compelling
clinical and financial reasons to better manage CMV drug resistance.

**Monitoring CMV drug resistance**

Unlike many pathogenic bacteria, it is time consuming and expensive to culture CMV
and test its drug-resistance phenotype by plaque assay. Fortunately, there is a growing
catalogue of known drug resistance mutations [7, 8] in the genes targeted by
ganciclovir, foscarnet and cidofovir: UL54 and UL97.

The primary approach to genetically confirming a clinical suspicion of antiviral
resistance has been to PCR amplify and Sanger sequence small regions of these
genes, which can be achieved directly from clinical samples. This method has
successfully identified hundreds of mutations which may convey resistance to one or
more drugs (and their prodrugs or oral derivatives). Traditional small sequencing of
these PCR products is relatively inexpensive, but cannot be easily scaled over the
growing number of genes, widely distributed over CMV’s large (~230kb) genome, on which new anti-CMV drugs act. It is also limited to detecting resistance mutations which reach frequencies of greater than ~20%.

PCR amplification of CMV fragments has increasingly being combined with high-throughput sequencing technologies. Amplicons are sequenced to high depth using a variety of next-generation sequencing platforms. This approach detects resistance mutations sooner, at lower frequencies, than Sanger sequencing of PCR products, with studies reporting detection of resistance mutations at frequencies of 3% [9, 10]. Unfortunately, as with Sanger sequencing, this approach still relies on labour-intensive PCR for the growing number of genes targeted by new antivirals and other therapies.

Novel approaches are being developed which can sequence whole CMV genomes, or genes of interest, without the need for PCR or virus isolation and culture. Using technology developed for human whole-exome capture and pull down, methods such as SureSelect (Agilent) or SeqCap (Nimblegen) can be used to sequence virus genomes to high depth directly from clinical samples. SureSelect uses custom baits to capture CMV sequences and enrich them in the DNA that forms a sequencing library. This approach has been very successful in sequencing relatively low virus load samples for other herpesviruses [11, 12], and allows antiviral resistance mutations to be monitored even as circulating CMV populations in blood, urine or CSF decline to low levels.

The future of CMV disease management

It is recognised that there is no unified strategy in place for monitoring the emergence of CMV drug resistance in immunocompromised paediatric patients [13]. Clinical trials of antiviral drugs are typically performed in adults rather than children. Choosing which drugs to treat with, for what duration and dose, and managing clinically suspected drug resistance is therefore even more challenging in paediatric patients than in adults [14].

Further studies are needed to establish at what frequency a resistance mutation must be present to warrant clinical action. Published data suggests that resistance mutations seen at high frequencies at the point of clinical diagnosis of resistance can be detected weeks earlier by deep sequencing, at frequencies <10% [10]. Researchers and
clinicians need data on whether, in the presence of a low-frequency drug resistance mutation, modifying an existing treatment schedule to increase the dosage or change drug delivery method to achieve better tissue penetration is a viable treatment option.

Deep-sequencing studies of the evolution of antiviral resistance in CMV disease also highlight the need for new drugs which target different parts of the CMV genome to avoid the emergence of cross-resistant variants. Given the occurrence of single nucleotide variants which convey resistance to more than one drug, multi-drug resistant CMV is a known phenomenon [10]. At present, clinical sequencing may reveal that a patient's circulating CMV strain or strains are already resistant to all available treatment options. In the future, if drugs such as artesunate, leflunomide and letermovir are more widely used [15], sequence data could inform a clinical decision to switch to drugs which act on different CMV genes. By using these new drugs, clinicians may be able to avoid the problem of pre-existing cross-resistance in previously treated patients, a significant problem for drugs which target UL54 and UL97.

Adoptive immunotherapy is a further weapon in the CMV management arsenal, infusing the host with CMV-specific cytotoxic T cells, and it may one day reduce reliance on antivirals [16]. It is also likely to drive CMV evolution, selecting for CMV epitopes which escape the infused T cells. These mutations will expand the number of genes which need to be sequenced in order to manage CMV infection in immunocompromised patients. It will rapidly become intractable to use PCR-based methods of CMV sequencing, if this point has not already been reached. The future of CMV management will require whole-genome sequencing and analysis of circulating CMV strains within each patient. This will in turn create a need for automated, scalable solutions for monitoring CMV genome variation, as the current bioinformatic burden of sequence analysis is a limiting factor in reducing the turn-around times from sample to personalised drug-resistance profile.

Future perspective

While the majority of immunocompromised paediatric patients with CMV infections are successfully treated with standard approaches to CMV load monitoring (qPCR) and
prophylactic or preemptive antiviral treatment, clinicians cannot currently predict which patients will fail first-line therapies due to pre-existing resistance mutations or progress to multidrug-resistant CMV infections. Prospective and regular sequencing of CMV, particularly in patients who have already been treated with drugs with anti-CMV activity, is likely to become the new gold standard in virological monitoring within the near future. To achieve this goal, existing sequencing and analysis methods must be made scalable and automatable to reduce costs and turn-around times, and bring this technology into a healthcare laboratory setting.

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Abbreviations

CNS  Central nervous system
CMV  Cytomegalovirus
CSF  Cerebrospinal fluid
HCST  Haemopoetic stem cell transplant
HIV  Human immunodeficiency virus
PCR  Polymerase chain reaction
qPCR  Quantitative polymerase chain reaction

References (16/20 maximum)


