Temporal and spatial compartmentalization of drug-resistant CMV in a child with CMV meningoencephalitis: implications for sampling in molecular diagnosis.

Running tile: Drug-resistant CMV meningoencephalitis

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Abstract

We describe a case of antiviral-resistant cytomegalovirus meningoencephalitis occurring after hematopoietic stem cell transplantation. Antiviral-resistant cytomegalovirus was identified in blood 16 months earlier. However, wild-type cytomegalovirus was evidenced in blood when the meningoencephalitis was diagnosed. Treatment of meningoencephalitis should be adapted to all previously identified resistance mutations in any compartment.

Key words: cytomegalovirus; CMV; meningoencephalitis; antiviral resistance; UL97; UL54; ganciclovir; foscarnet; artesunate.
Case report

In July 2008, a 21 month-old patient was diagnosed with an inherited immune deficiency, a major histocompatibility complex (MHC) class II expression deficiency caused by homozygous mutation in the RFXANK gene. He had a history of successive herpes simplex virus type 1 (HSV-1) gingivostomatitis successfully treated with acyclovir (ACV). Positive human cytomegalovirus (CMV) serology suggested previous undiagnosed episode(s) of CMV viremia, which spontaneously resolved. At diagnosis (July 2008), an intravenous polyvalent immunoglobulins replacement and an oral ACV-based prophylaxis against HSV-1 infections were implemented.

In June 2009, an episode of asymptomatic CMV viremia was treated with intravenous ganciclovir (GCV). In July 2009, the patient received allogeneic hematopoietic stem cell transplantation (HSCT) using a HLA-mismatched unrelated cord blood unit and a conditioning regimen associating fludarabin, melphalan and alemtuzumab. At day 3 post-HSCT, a CMV disease was suspected because of concomitant diarrhoea, fever, and high CMV viremia. Because intravenous foscarnet (FOS) (90 mg/kg twice daily) failed to control the CMV viremia, GCV was added (5 mg/kg twice daily). FOS was replaced with cidofovir (CDV) between September and December 2009 (in addition to GCV) because of the diagnosis of a concomitant adenovirus infection. In November 2009, a CMV genotypic resistance test was performed by UL97 and UL54 viral gene direct sequencing (as previously described [1]), but no resistance mutation was evidenced (Table 1). The monitoring of GCV plasma exposure revealed extremely low trough GCV levels (< 0.1mg/L); adequate pharmacokinetic exposure (trough GCV level ≥ 0.5 mg/L) was achieved by increasing the daily GCV doses to 3 mg/kg/6h. Finally, because of the persistence of uncontrolled CMV viremia despite GCV + FOS bitherapy, artesunate was added in December 2009. CMV viremia resolved in January 2010 and the antiviral multitherapy was replaced with a valganciclovir (VGCV)-based
maintenance treatment. At the same time, HSCT graft rejection was diagnosed in our patient (MHC class II expression <3% in T lymphocytes).

In March 2010, the patient experienced a new episode of CMV viremia. CMV genotypic resistance test showed the following mutations: M460I in UL97 phosphotransferase, conferring resistance to GCV, and L545S in UL54 DNA polymerase, conferring resistance to GCV and CDV. However, CMV viremia could be promptly controlled with a GCV + FOS treatment.

In January 2011, a new episode of CMV viremia occurred under VGCV-based maintenance treatment. The genotypic resistance test showed the repopulation of the blood with wild-type strains (Table 1). After a 2-week-FOS therapy, which successfully controlled the viremia, a treatment with valaciclovir (VACV) was initiated - instead of VGCV - because of the risks of hematological toxicity in patients with prolonged VGCV exposure.

In September 2011, a rapidly progressive vision loss was diagnosed. The ophthalmological examination found bilateral pale papilla, without signs of viral retinitis. Cerebral magnetic resonance imaging showed abnormalities consistent with encephalitis. CMV PCR was negative in blood but positive (3.8 log_{10} copies/mL) in the cerebrospinal fluid (CSF). CMV genotypic resistance test performed in CSF sample evidenced H520Q and L545S mutations in UL97 phosphotransferase and UL54 DNA polymerase, respectively, conferring resistance to GCV and CDV. A GCV + FOS bitherapy failed either to improve the clinical symptoms or decrease the CMV viral load (VL) in CSF (3.9 log_{10} copies/mL), despite adequate GCV pharmacokinetic exposure. Because of the lack of efficacy of the antiviral treatment and its poor hematological and renal tolerance (deep neutropenia requiring daily filgrastim administration and severe tubulopathy), the GCV + FOS bitherapy was stopped in October 2011. Because (i) no other virologically-active antiviral drug to treat pediatric CMV disease was available in France at this time and (ii) the severe inherited immune deficiency of our
patient required a prophylaxis against HSV-1 infection, a VACV-based prophylaxis was implemented. Nine weeks later, the patient presented with CMV viremia (3.4 log_{10} copies/mL). CMV genotypic resistance test showed discrepant results in blood and CSF: whereas a resistant strain still predominated in CSF (H520Q and L545S mutations), a wild-type virus was identified in blood. CMV resistance was retrospectively assessed again in the previously tested blood sample: this second test isolated a resistant strain (H520Q and L545S mutations). A GCV + FOS bitherapy was started again but failed to decrease the CMV VL in blood or CSF. During subsequent months, CMV genotypic resistance tests were performed in blood samples and showed M460I and L545S mutations in UL97 phosphotransferase and UL54 DNA polymerase, respectively (Table 1).

Because of the absence of available virologically-active antiviral drugs and the progressive development of a refractory aplastic anemia, it was decided to proceed to a second HSCT in our patient as an attempt to cure its inherited immune deficiency. Unfortunately, the child died from multivisceral failure 10 days after a second HSCT attempt.

CMV is one of the clinically most significant viral pathogens causing infections in immunocompromised patients, especially in HSCT recipients. In the transplant setting, the emergence of CMV resistance to antivirals, favoured by long-term exposure to antiviral drugs and profound immunodeficiency, constitutes a rising therapeutic challenge [2]. CMV infection of the central nervous system (CNS) is rare in HSCT recipients. Fifteen well-documented cases have been reported [2-13]. In most of them, the authors described a high VL in the CSF contrasting with low peripheral blood VL [2, 9, 13] or low antigenemia [3, 4], suggesting a high level of viral replication in the CNS. In this study, we have observed a temporal and spatial evolution of mutations in the UL97 and UL54 genes of CMV in a child.
with severe inherited immune deficiency, who developed a CMV disease refractory to
treatment with both GCV and FOS.

The most striking result was the spatial compartmentalization of CMV mutations in this
patient. Indeed, whereas the CMV genotypic resistant tests did not reveal any resistance
mutation in blood samples in January 2011 and February 2012, indicating a repopulation of
the blood compartment by wild-type strains, the patient developed in September 2011 a CMV
meningoencephalitis due to a multidrug-resistant strain. Two hypotheses could explain these
findings. First, the resistant strain isolated in CSF in 2011 could have been recently selected
because of the low CNS penetration of the antiviral prophylaxis. At the time of diagnosis of
CMV meningoencephalitis, the child had being treated with VACV prophylaxis for 9 months.

Few previous retrospective reports supported selection of mutations conferring GCV/ACV
cross-resistance [14-16]. Further large prospective studies are needed to accurately assess the
risk of selecting CMV mutations in \textit{UL97} and/or \textit{UL54} genes under ACV/VACV prophylaxis.

Second, prolonged exposure to antivirals between 2009 and 2011 (especially during the long
episode of uncontrolled viremia under treatment in 2009-2010) could have contributed to the
selection of a resistant strain in blood, which may have fuelled the CNS and persisted in this
compartment during a long asymptomatic period before a late symptomatic reactivation. No
strain with H520Q (\textit{UL97} gene) and L545S (\textit{UL54} gene) mutations has been detected in blood
between June 2009 and September 2011. However, we cannot exclude that such mutant virus
has been selected several months/years prior to its detection and persisted in blood and/or
CSF as a minor viral quasispecies. Whatever the hypothesis, our results show that blood
samples may not be representative for the resistance status of the virus isolated in the CNS
compartment. Interestingly, previous reports of CMV CNS diseases which compared the
CMV genotypic resistance profiles in concomitant CSF and blood samples, described similar
profiles in both compartments [6, 11], or viral compartmentalization with wild-type CMV in
CSF samples and resistant CMV in blood and other sites. [5, 8, 9, 13] The authors suggested that decreased CMV fitness due to drug-resistant mutations located in UL97 and/or UL54 genes could explain the predominance of wild-type strains in the CSF, even in cases of previous selection of resistant mutants in blood [17]. This is the first report describing that meningoencephalitis due to resistant CMV can occur even in case of recent isolation of wild-type strains in blood.

Three patterns of UL97 and UL54 mutations were observed in the successive blood specimens: (i) no drug-resistant mutations, (ii) M460I/L545S and (iii) H520Q/L545S. Although CMV genome were not full-length characterized in our patient, the same natural polymorphisms located in UL97 phosphotransferase (amino acid changes = Q19E, N68D, S108N and I244V) and UL54 DNA polymerase (amino acid changes = A885T and N898D) were observed in all strains. Consequently, these viruses may represent different evolution patterns under antiviral pressure of the viral quasispecies derived from a unique virus.

Because population-based sequencing could only detect the viral variants present at more than 20% of the total viral population, minor variants may not have been isolated. Indeed, the resistant strain harbouring the M460I mutation, which was evidenced in blood in May 2010, was not detected in January 2011 and February 2012. However, this mutation was again detected in blood in May/June 2012, indicating the long-term persistence of this archived resistant strain among the blood quasispecies. Moreover, the retrospective reanalysis of the blood sample collected in February 2012 showed 2 different results of resistance patterns: a wild-type strain in the first case, a virus with H520Q/L545S mutations in the other case, indicating the cocirculation of distinct quasispecies harbouring different mutations in blood.

Our findings could have a major impact for management of CMV disease. Indeed, this study (i) confirms that analysis of concomitant blood samples cannot predict the genotypic resistance profile of strains in the CSF, (ii) indicates that CMV molecular resistance
monitoring should be performed in all body compartments where CMV is present or suspected, (iii) suggests that the initial antiviral treatment of CMV CNS disease should be adapted to all previously identified viral resistance mutations in any body compartment, and (iv) suggests that monitoring of CMV molecular resistance should be performed frequently, especially in case of long exposure to antiviral drugs, because of the lack of sensitivity of CMV gene direct sequencing for the detection of minor resistant variants.

We can highly suspect that the resistance mutations have been selected in our patient during the prolonged uncontrolled viremia in 2009-2010. Several factors have been previously reported as being associated with the selection of resistant strains, especially a high CMV VL, a prolonged exposure to GCV and suboptimal plasma GCV levels [2, 18]. All of these risk factors were present in our patient, especially the long exposure to antiviral treatments. Interestingly, dramatically low trough GCV levels were observed, despite having used the GCV recommended daily doses (i.e. 5mg/kg/12h). Although GCV is widely used to treat CMV infection in immunocompromised children, previous pediatric pharmacokinetics reports about GCV exposure are sparse and heterogeneous. Further prospective studies are urgently needed, especially in order (i) to assess that the currently recommended GCV daily doses are appropriate in the pediatric population and (ii) to study if a monitoring of GCV plasma levels should be recommended in children in order to avoid the selection of resistance mutations.
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References


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<thead>
<tr>
<th>Dates</th>
<th>Blood</th>
<th>Cerebrospinal fluid</th>
<th>Antiviral treatment</th>
<th>CMV PCR ^</th>
<th>Amino acid changes associated with CMV resistance to antiviral drugs</th>
<th>CMV PCR ^</th>
<th>Amino acid changes associated with CMV resistance to antiviral drugs</th>
<th>Drug</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 2008 – June 2009</td>
<td>-</td>
<td>-</td>
<td>oral ACV</td>
<td>11 months</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 2009</td>
<td>+</td>
<td>-</td>
<td>GCV</td>
<td>2 weeks</td>
<td><em>(3.9 → &lt;2.7)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>June – July 2009</td>
<td>-</td>
<td></td>
<td>oral ACV</td>
<td>4 weeks</td>
<td><em>(3.2 →&lt;5.4)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>July – August 2009</td>
<td>+</td>
<td><em>(5.4 →&lt;5.9)</em></td>
<td>FOS</td>
<td>3 weeks</td>
<td><em>(1.9 →&lt;3.6)</em></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>August – Sept. 2009</td>
<td>+</td>
<td><em>(5.4 →&lt;5.9)</em></td>
<td>FOS + GCV</td>
<td>3 weeks</td>
<td><em>(1.6 →&lt;5.8)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Sept. – December 2009</td>
<td>+</td>
<td><em>(3.9 →&lt;3.6)</em> Nov. 2009</td>
<td>CDV + GCV</td>
<td>14 weeks</td>
<td><em>(5.4 →&lt;3.6)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>December 2009</td>
<td>+</td>
<td><em>(3.9 →&lt;3.6)</em></td>
<td>FOS + GCV</td>
<td>2 weeks</td>
<td><em>(3.6 →&lt;2.7)</em></td>
<td></td>
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<tr>
<td>Dec. 2009 – Jan. 2010</td>
<td>+</td>
<td><em>(5.8 →&lt;2.7)</em></td>
<td>FOS + GCV + artesunate</td>
<td>4 weeks</td>
<td><em>(2.9 →&lt;4.4)</em></td>
<td></td>
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</tr>
<tr>
<td>January – March 2010</td>
<td>-</td>
<td></td>
<td>VGCV</td>
<td>2 months</td>
<td><em>(4.4 →&lt;2.7)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>March – April 2010</td>
<td>+</td>
<td><em>(2.9 →&lt;4.4)</em></td>
<td>VGCV</td>
<td>2 months</td>
<td><em>(1.6 →&lt;5.8)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>May 2010</td>
<td>+</td>
<td><em>(3.9 →&lt;3.6)</em></td>
<td>FOS + GCV</td>
<td>2 weeks</td>
<td><em>(1.6 →&lt;5.8)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>June 2010 – Jan. 2011</td>
<td>-</td>
<td></td>
<td>VGCV</td>
<td>7 months</td>
<td><em>(3.3 →&lt;2.7)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>January 2011</td>
<td>+</td>
<td><em>(3.3 →&lt;2.7)</em></td>
<td>FOS</td>
<td>2 weeks</td>
<td><em>(3.8 →&lt;3.9)</em></td>
<td></td>
<td></td>
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<tr>
<td>January – Sept. 2011</td>
<td>-</td>
<td></td>
<td>VACV</td>
<td>9 months</td>
<td><em>(3.8 →&lt;3.9)</em></td>
<td></td>
<td></td>
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<tr>
<td></td>
<td></td>
<td><em>L548S</em></td>
<td></td>
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</table>
### Table 1. Evolution of the cytomegalovirus (CMV) PCR and CMV genotypic resistance tests in blood and cerebrospinal fluid samples.

<table>
<thead>
<tr>
<th>Time Period</th>
<th>PCR Result</th>
<th>UL97 Changes</th>
<th>UL54 Changes</th>
<th>Antivirals</th>
<th>Duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oct. – Nov. 2011</td>
<td>+ (3.9 → 4.9)</td>
<td>-</td>
<td>-</td>
<td>VACV</td>
<td>9 weeks</td>
</tr>
<tr>
<td>Nov. 2011 – June 2012</td>
<td>+ (3.4 → 3.7)</td>
<td>0</td>
<td>0</td>
<td>VACV</td>
<td>7 months</td>
</tr>
<tr>
<td>Feb. 2012 §</td>
<td>0</td>
<td>H520Q</td>
<td>L545S</td>
<td>VACV</td>
<td></td>
</tr>
<tr>
<td>Feb. 2012 §</td>
<td>0</td>
<td>M460I</td>
<td>L545S</td>
<td>FOS + GCV</td>
<td>5 weeks</td>
</tr>
<tr>
<td>May 2012</td>
<td>0</td>
<td>M460I</td>
<td>L545S</td>
<td>VACV</td>
<td></td>
</tr>
<tr>
<td>June – Aug. 2012</td>
<td>+ (3.7 → 5.3)</td>
<td>M460I</td>
<td>L545S</td>
<td>FOS + GCV</td>
<td>5 weeks</td>
</tr>
<tr>
<td>(death)</td>
<td></td>
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</tbody>
</table>

CMV = cytomegalovirus; UL97 PT = UL97 phosphotransferase; UL54 DP = UL54 DNA polymerase; ACV = acyclovir; GCV = intravenous ganciclovir; FOS = foscarnet; CDV = cidofovir; VGCV = valganciclovir; VACV = valaciclovir; i.v. = intravenous

^ In case of positive CMV PCR, results of the PCR at the initiation and at the end of each antiviral regimen were indicated in parenthesis (expressed in log_{10} copies/mL).

* In all genotypic resistance tests, the following amino acid changes relative to natural polymorphisms of CMV UL97 phosphotransferase were isolated: Q19E, N68D, S108N, I244V.

** In all genotypic resistance tests, the following amino acid changes relative to natural polymorphism of CMV UL54 DNA polymerase were isolated: A885T, N898D.

§ Genotypic resistance tests performed in the same blood sample (the second test has been retrospectively assessed).