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Case report

Ganciclovir-resistant cytomegalovirus encephalitis in a hematopoietic stem cell transplant recipient

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We describe a 41-year-old patient, who upon receiving a bone marrow transplant in order to treat chronic myeloid leukemia, developed cytomegalovirus (CNV) retinitis and encephalitis under the ganciclovir maintenance treatment. Analysis of sequential viral isolates recovered from the patient's cerebrospinal fluid and blood showed CMV DNA with a UL97 mutation (M460V) known to confer ganciclovir resistance. Foscarnet resistance mutations were not found. Although therapy was switched to foscarnet when ganciclovir resistance was suspected, the patient was lost on posttransplant day 200. *Journal of NeuroVirology* (2010) **16,** 174–178.

Keywords: bone marrow transplantation; encephalitis; cytomegalovirus; ganciclovir resistance; UL97

Introduction

Cytomegalovirus (CMV) encephalitis occurs rarely in bone marrow transplant recipients. Here, we describe a patient under preemptive therapy developing ganciclovir resistance in UL97 gene, resulting in cytomegalovirus encephalitis and retinitis.

Case report

A 41-year old CMV-seronegative patient underwent allogeneic stem cell transplantation from his CMV-seropositive brother for chronic phase chronic myeloid leukemia in July 2006. He received busulfan and cyclophosphamide as a conditioning regimen, a short course of methotrexate and cyclosporine as prophylaxis for graft-versus-host disease (GVHD), and prophylactic acyclovir from pretransplant day 8 to posttransplant day 30. Maculopapular rash was observed on posttransplant day 11 and GVHD was shown on skin biopsy. On posttransplant day 14, steroid therapy 2 mg/kg/day was started. Thereafter, skin manifestations progressed to grade III GVHD. On posttransplant day 39, steroid dose was tapered because of steroid-related myopathy and mycophenolate mofetil 2 × 1000 mg was added to the therapy.

CMV reactivation was detected by polymerase chain reaction (PCR) (viral load, 3600 copies/ml of whole blood) on posttransplantation day 46. According to our protocol, preemptive antiviral therapy with intravenous ganciclovir (GCV) was initiated (induction, 5 mg/kg twice daily for 2 weeks). At posttransplant day 60, viremia was cleared (<400 copies/ml of whole blood) and GCV maintenance treatment was applied as 5 mg/kg once daily, on weekdays only.

On posttransplant day 80 (day 20 of GCV maintenance), CMV viremia rebounded to 1010 copies/ml and 5 days later the patient became drowsy and apathic. On posttransplant day 90, the patient developed blurry vision, diplopia, and aphasia. A fundoscopic examination revealed retinitis. A contrast-enhanced magnetic resonance imaging (MRI) of the head showed periventricular enhancement, ventricular enlargement, and T2-hyperintense white matter lesions (Figure 1). Lumbar puncture was performed on posttransplant day 96. Analysis of cerebrospinal fluid (CSF) revealed 0 cells/ml, 96 mg/dl protein, and 72 mg/dl glucose. CSF PCR was negative for CMV, tuberculosis, and toxoplasmosis. GCV treatment (2 × 5 mg/kg) was continued until day 130.

CMV viremia showed another peak (8660 copies/ml) on posttransplant day 129, and therapy was

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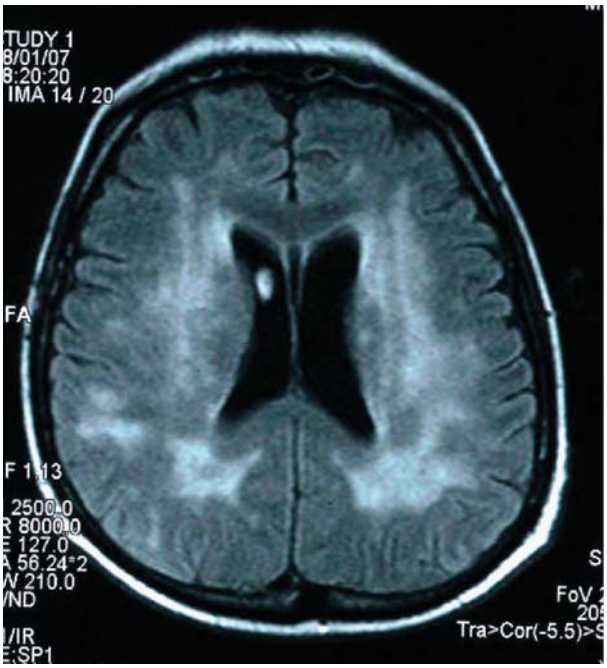

Figure 1 Contrast-enhanced MRI of the head indicating periventricular enhancement, ventricular enlargement and T2 hyperintense white matter lesions.

changed to foscarnet (60 mg/kg, three times per day) due to clinical suspicion of GCV resistance. Meanwhile, the patient's condition was well except his skin rashes because of GVHD. CMV PCR was found negative on day 151. However, the patient became increasingly withdrawn, apathetic, and mute within the next 14 days. PCR performed on a blood sample on day 170 was borderline positive for CMV (386 copies/ml). Hallucinations, pansinusitis, and paralytic ileus episodes developed during the follow-up period. In MRI, partially symmetric, coalescing, patchy T2-hyperintense lesions located at the subcortical and deep periventricular white matter were detected.

CSF performed on day 175 showed 300 cells/ml (97% neutrophils), 46 mg/dl protein, and 77 mg/dl glucose (serum glucose: 98 mg/dl). In the CSF sample, PCR results for herpes simplex virus (HSV), Epstein-Barr virus (EBV), varicella-zoster virus (VZV), human herpesvirus (HHV)-6, and JC virus were negative. Bacterial and fungal cultures were also negative. However, qualitative PCR for CMV was positive in CSF. Sequence analysis of CMV DNA from both blood and CSF samples revealed polymorphisms in UL97 gene (M460V), which is known to confer GCV resistance.

Despite foscarnet therapy, the patient's mental status did not improve and developed grand mal seizures progressing to status epilepticus and was entubated for respiratory failure. Imaging studies revealed nodular consolidation in the lung and culture of bronchoalveolar liquid (BAL) was positive for *Aspergillus fumigatus*. Voriconozole (600 mg, twice daily) was added to therapy but the patient was lost on posttransplant day 200. The clinical progress and CMV quantitation are summarized in Figure 2.

Discussion

The main clinical manifestations of CMV disease after bone marrow transplantation (BMT) are interstitial pneumonitis, esophagitis, and enteritis. Retinitis and encephalitis are extremely rare. The reasons for the low incidence of retinal and central nervous system (CNS) disease in patients undergoing BMT is unknown. However, the present case and other recently reported cases of CMV encephalitis may be indicative of increasing rates.

CMV encephalitis cases reported in the literature are summarized in Table 1 (Hamprecht *et al*, 2003; Julin *et al*, 2002; Miller *et al*, 2006; Seo *et al*, 2001; Wolf *et al*, 2003). The present case and its comparison with other six cases give medical clues on drug-resistant CMV infection of the CNS in BMT recipients (Hamprecht *et al*, 2003; Julin *et al*, 2002; Miller *et al*, 2006; Seo *et al*, 2001; Wolf *et al*, 2003). All cases had CMV ventriculoencephalitis and pathologic findings were described in one of them (Miller *et al*, 2006). CMV ventriculoencephalitis often follows CMV retinitis as a late complication in acquired immunodeficiency syndrome (AIDS) patients, despite continuous antiviral treatment (Berman and Kim, 1994; Holland *et al*, 1994; Kalayjian *et al*, 1993). Three of seven stem cell recipients with CMV infection of the CNS were associated with retinitis. In the present case, retinitis symptoms developed while the patient was on GCV treatment (5 mg/kg; once-daily dose) and diagnosis was confirmed by visualization of periventricular enhancement in contrast-enhanced MRI. Similarly, Seo *et al* (2001) reported a patient with CMV retinitis, who subsequently developed encephalitis despite the administration of ganciclovir (5 mg/kg q12h) and intravenous immunoglobulin G (IV IgG) (400 mg/q48h). Hamprecht *et al* (2003) have also noted that retinitis developed along with encephalitis in their case. Although CMV retinitis is not a prerequisite for CNS involvement in BMT patients, it may potentially increase the risk of subsequent ventriculoencephalitis. Fundoscopic changes in patients with risk must be followed up periodically.

CMV serology is an important indicator of CMV reactivation and it is sufficient for only the donor (as in our case and the case described by Hamprecht *et al* [2003]) to be CMV positive for detection of posttransplantation viremia. In addition, granulocyte infusions obtained from seropositive donors may also be a source of CMV as in case 2 reported by Wolf *et al* (2003).

Invasive aspergillosis is an increasingly common and often fatal opportunistic fungal infection in

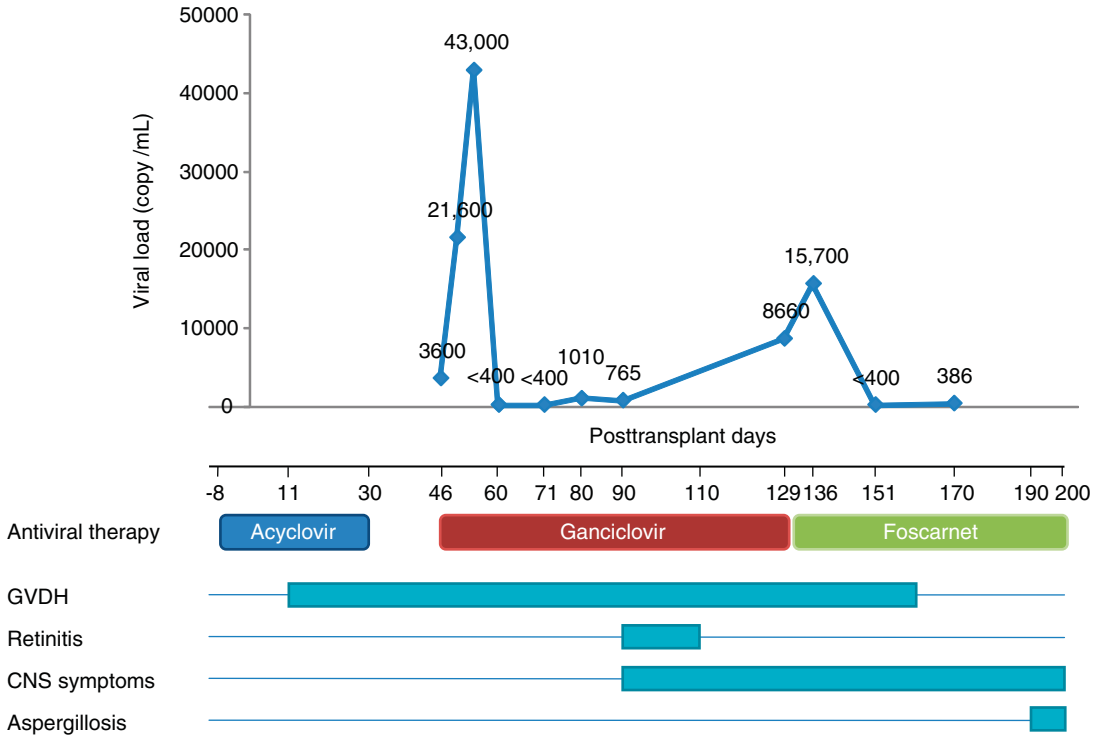

Figure 2 CMV viral load, antiviral treatments, and clinical course of the present case. Patient was lost on posttransplant day 200. Graphic representation was modified from Julin *et al* (2002).

Table 1 Clinical and laboratory data of ganciclovir-resistant encephalitis patients in the literature

	Disease	CMV serology	Retinitis	Invasive mycose	GVHD	Drug resistance
Hamprecht *et al* (2003)	AML	D + R −	+	—	NA	UL97 mutation
Julin *et al* (2002)	MLD	D − R +	NA	*Aspergillus fumigatus*	Acute	UL97 mutation
Miller *et al* (2006)	AML	D + R +	NA	*Aspergillus fumigatus*	Acute	UL54 mutation
Seo *et al* (2001)	ALL	D + R +	+	*C. bertholletiae*	Chronic	UL54 mutation
Wolf *et al* (2003)						
Case 1	AML	D + R +	NA	—	Chronic	UL97-UL54 mutation
Case 2	AML	D − R −	NA	—	Chronic	UL97-UL54 mutation
Present case	CML	D + R −	+	*Aspergillus fumigatus*	Acute	UL97 mutation

Note. CMV, cytomegalovirus; GVHD, graft-versus-host disease; AML, acute myeloid leukemia; D, donor; R, recipient; MLD, metachromatic leukodystrophy; NA, data not available; ALL, acute lymphoid leukemia; CML, chronic myeloid leukemia.

patients with hematologic malignancies. Some predictive factors such as prolonged corticosteroid therapy, GVHD, and CMV infection are important risk factors for the recurrence and progression of *Aspergillus* infections after bone marrow recovery (Marr *et al*, 2002). Four of seven stem cell recipients with CMV infection were complicated by invasive mycoses (Table 1). Invasive pulmonary aspergillosis was seen in three cases including ours and renal abscess due to *Cunninghamella bertholletiae* was detected in one case (Julin *et al*, 2002; Miller *et al*, 2006; Seo *et al*, 2001). CMV pneumonitis was described in two patients.

There is no consensus regarding the best prophylactic antiviral regimen for CMV infection in patients with BMT. Our center has been using acyclovir for CMV prophylaxis. Acyclovir has relatively low potency against CMV *in vitro* and it can change the natural course of CMV reactivation after BMT (Meyers *et al*, 1988). However, the CMV disease cases discussed here suggest that prophylactic or preemptive treatment strategy may not be effective, especially in patients treated with immunosuppressive agents for GVHD. GVHD developed in all of the patients discussed here despite immunosuppressive therapies such as mycophenolate mofetil, cyclosporine, antithymocyte globulin, corticosteroids, thiotepa, and fludarabine. If the risk of GVHD is unusually high, e.g., in T-cell depletion with antithymocyte globulin for matched-unrelated or haploidentical BMT, treatment for CMV infection should be started after transplant as soon as possible. It has

been shown that CMV seropositivity is a strong negative prognostic factor in this setting and that preemptive anti-CMV strategies do not always improve outcome (Kroger *et al*, 2001). However, prophylactic ganciclovir administered along with GVHD treatment with corticosteroid-containing regimens was shown to be effective in one series (Verdonck *et al*, 1997).

There are four antiviral agents (ganciclovir, acyclovir, foscarnet, cidofovir) approved for clinical use for the treatment of CMV infection. Most CMV antiviral drugs' usage is directed at prevention of disease but treatment of disease remains only variably successful in the BMT recipient. Antiviral drug resistance has become a major problem in treatment of CMV infection in recent years. Resistance to currently available antiviral drugs may result from mutations in either the UL97 or the UL54 gene, or both. UL97 encodes the viral protein kinase responsible for the initial phosphorylation of ganciclovir and cidofovir. UL54 encodes the DNA polymerase, which is the target of ganciclovir, cidofovir, and foscarnet. Genotypic and phenotypic analysis of drug resistance in six patients reported in the literature reveals that CMV drug resistance mutations may play a role in pathogenesis. Unfortunately, progressive encephalitis may develop even when viral strains in the CNS are susceptible to antiviral drugs. Sequencing of both UL97 and UL54 were performed in the CNS isolates obtained from all patients. Mutation types are shown in Table 1. In our patient, ganciclovir-resistant CMV mutations were present in the viral DNA found in peripheral blood and CSF. Upon suspicion of resistance, foscarnet induction and maintenance therapy were administered and eventually CMV DNA viral load decreased in the blood. Despite this decline in the viral load, our patient's general condition did not improve. It seems that CMV viral load detected in blood may not adequately reflect viral replication in sequestered sites, especially CNS. Similar compartmentalization of viral replication was also noted in other cases (Julin *et al*, 2002; Miller *et al*, 2006). In our patient, blood viral load remained low while the encephalitis developed, suggesting selective viral replication and emergence of ganciclovir resistance in CNS. However, all CSF isolates remained susceptible to foscarnet, indicating that the observed ganciclovir resistance is not sufficient to explain the active viral replication in the CNS. Inefficient penetration of ganciclovir and foscarnet into the brain may be responsible for progression of the disease (Arribas *et al*, 1996; Mastroianni *et al*, 1994; McCutchan, 1995).

In conclusion, our experience suggests that CMV ventriculoencephalitis may also be seen in immunocompromised BMT patients. CMV disease may develop despite anti-CMV prophylaxis and routine preemptive therapy because of the emergence of resistance and, possibly, low penetration of antiviral drugs into the CNS. In cases of CNS involvement, antiviral monotherapy may not be enough for adequate clinical response. Therefore, combination antiviral therapy and alternative methods are needed for the treatment of CMV disease of the CNS. Novel immunotherapy techniques such as adoptive transfer of CMV-specific T cells (Leen *et al*, 2006) or active immunization of donor and recipient could be the future treatment alternatives to be used in CMV control.

Declaration of interest: The authors report no conflicts of interest. The authors alone are responsible for the content and writing of the paper.

References

Arribas JR, Storch GA, Clifford DB, Tselis AC (1996). Cytomegalovirus encephalitis. *Ann Intern Med* **125:** 577–587.

Berman SM, Kim RC (1994). The development of cytomegalovirus encephalitis in AIDS patients receiving ganciclovir. *Am J Med* **96:** 415–419.

Hamprecht K, Eckle T, Prix L, Faul C, Einsele H, Jahn G (2003). Ganciclovir-resistant cytomegalovirus disease after allogeneic stem cell transplantation: pitfalls of phenotypic diagnosis by in vitro selection of an UL97 mutant strain. *J Infect Dis* **187:** 139–143.

Holland NR, Power C, Mathews VP, Glass JD, Forman M, McArthur JC (1994). Cytomegalovirus encephalitis in acquired immunodeficiency syndrome (AIDS). *Neurology* **44**(3 Pt 1): 507–514.

Julin JE, van Burik JH, Krivit W, Webb C, Holman CJ, Clark HB, *et al* (2002). Ganciclovir-resistant cytomegalovirus encephalitis in a bone marrow transplant recipient. *Transpl Infect Dis* **4:** 201–206.

Kalayjian RC, Cohen ML, Bonomo RA, Flanigan TP (1993). Cytomegalovirus ventriculoencephalitis in AIDS. A syndrome with distinct clinical and pathologic features. *Medicine (Baltimore)* **72:** 67–77.

Kroger N, Zabelina T, Kruger W, Renges H, Stute N, Schrum J, *et al* (2001). Patient cytomegalovirus seropositivity with or without reactivation is the most important prognostic factor for survival and treatment-related mortality in stem cell transplantation from unrelated donors using pretransplant in vivo T-cell depletion with anti-thymocyte globulin. *Br J Haematol* **113:** 1060–1071.

Leen AM, Myers GD, Sili U, Huls MH, Weiss H, Leung KS, *et al* (2006). Monoculture-derived T lymphocytes specific for multiple viruses expand and produce clinically relevant effects in immunocompromised individuals. *Nat Med* **12:** 1160–1166.

Marr KA, Carter RA, Boeckh M, Martin P, Corey L (2002). Invasive aspergillosis in allogeneic stem cell transplant recipients: changes in epidemiology and risk factors. *Blood* **100:** 4358–4366.

Mastroianni CM, Ciardi M, Folgori F, Sebastiani G, Vullo V, Delia S, *et al* (1994). Cytomegalovirus encephalitis in two patients with AIDS receiving ganciclovir for cytomegalovirus retinitis. *J Infect* **29:** 331–337.

McCutchan JA (1995). Clinical impact of cytomegalovirus infections of the nervous system in patients with AIDS. *Clin Infect Dis* **21**(Suppl 2): S196–S201.

Meyers JD, Reed EC, Shepp DH, Thornquist M, Dandliker PS, Vicary CA, *et al* (1988). Acyclovir for prevention of cytomegalovirus infection and disease after allogeneic marrow transplantation. *N Engl J Med* **318:** 70–75.

Miller GG, Boivin G, Dummer JS, McConnell T, Becher MW, Kassim A, *et al* (2006). Cytomegalovirus ventriculoencephalitis in a peripheral blood stem cell transplant recipient. *Clin Infect Dis* **42:** e26–e29.

Seo SK, Regan A, Cihlar T, Lin DC, Boulad F, George D, *et al* (2001). Cytomegalovirus ventriculoencephalitis in a bone marrow transplant recipient receiving antiviral maintenance: clinical and molecular evidence of drug resistance. *Clin Infect Dis* **33:** e105–e108.

Wolf DG, Lurain NS, Zuckerman T, Hoffman R, Satinger J, Honigman A, *et al* (2003). Emergence of late cytomegalovirus central nervous system disease in hematopoietic stem cell transplant recipients. *Blood* **101:** 463–465.

Verdonck LF, Dekker AW, Rozenberg-Arska M, van den Hoek MR (1997). A risk-adapted approach with a short course of ganciclovir to prevent cytomegalovirus (CMV) pneumonia in CMV-seropositive recipients of allogeneic bone marrow transplants. *Clin Infect Dis* **24:** 901–907.

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