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Ganciclovir-Resistant Cytomegalovirus (CMV) Retinitis in a Patient with Wild-Type CMV in Her Plasma

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A patient with systemic cytomegalovirus (CMV), including chorioretinitis, received localized and systemic ganciclovir, systemic cidofovir analog, and localized foscarnet. Mutations conferring ganciclovir and cidofovir resistance were detected in CMV from the aqueous fluid but not in CMV from plasma. Quantifying CMV from aqueous fluid was valuable for monitoring the clinical response and predicting resistance.

CASE REPORT

A 39-year-old Asian female with Burkitt's lymphoma was completing therapy with hyperfractionated rituximab, cyclophosphamide, vincristine, doxorubicin, and dexamethasone when she presented with neurological symptoms. Cytomegalovirus (CMV) was detected by a laboratory-developed PCR test (LDT) (12) from her plasma and cerebrospinal fluid (CSF), and intravenous (i.v.) ganciclovir was initiated. The ophthalmology service noted that although she had no visual symptoms, the patient had a small focus of CMV chorioretinitis nasally in the right eye and a larger lesion in the periphery of the left eye. Due to a national foscarnet shortage, she was treated with bilateral intravitreal ganciclovir injections (0.5 mg). Concurrent anterior chamber paracenteses were performed, and aqueous fluid samples from both eyes were positive for CMV by the LDT. After 11 days, her systemic therapy was switched to an investigational cidofovir analog (CMX001; Chimerix, Durham, NC) due to a lack of clinical improvement and increasing CMV loads in plasma. After 10 days on CMX001, she was switched back to i.v. ganciclovir because she developed ileus. After approximately 2 weeks of i.v. ganciclovir and decreasing CMV loads in plasma, she was transitioned to oral valganciclovir. Although her initial bilateral retinitis clinically responded rapidly to a single ganciclovir injection and her systemic therapy as described above, she showed evidence of recurrence in her right eye approximately 2 months after initial presentation (Fig. 1B). This recurrence initially responded to a repeat ganciclovir injection, but genotyping performed from the aqueous sample revealed a mixture of wild-type CMV and a population with a UL97 gene mutation (C603R) conferring ganciclovir resistance. The plasma was tested concurrently and showed only a wild-type population of CMV. Once foscarnet became available, she was given intravitreal injections (2.4 mg) every 2 weeks in her right eye, until another recurrence about 4 months after initial presentation which led to twice-weekly injections of foscarnet (Fig. 1C). Repeat CMV genotyping from her right aqueous fluid revealed yet another population of CMV with a UL54 gene mutation (T503I) conferring resistance to both cidofovir and ganciclovir. Having received a total of 43 injections in her right eye, the patient was stable at 9 months from initial presentation. Her left eye remained stable after the initial ganciclovir injection and a consolidation foscarnet injection at 3 months postpresentation, but it showed evidence of recurrence at approximately 6 months postpresentation (Fig. 2B). She received twice-weekly foscarnet injections in the left eye and was stable at 9 months postpresentation. She received a total of 36 injections in her left eye. She has not exhibited any evidence of toxicity related to intravitreal injections. Throughout her course, quantitative real-time PCR of aqueous fluid obtained from weekly anterior chamber paracenteses was used to monitor her response to therapy (Fig. 1 and 2, lower panels).

CMV chorioretinitis is a major cause of morbidity among immunocompromised patients. Although antiviral medications (ganciclovir, foscarnet, and cidofovir) provide effective treatment options for most patients, phenotypic resistance to anti-CMV medications is reportedly 5 to 25% (3, 7). Sequencing of the CMV UL97 (phosphotransferase) and UL54 (DNA polymerase) genes for mutations known to confer resistance is performed to predict drug resistance to ganciclovir and all three drugs (ganciclovir, foscarnet, and cidofovir), respectively (1). Many documented UL97 and UL54 gene mutations have now been reported in the literature, with various degrees of phenotypic resistance (2, 5, 10). The UL97 C603R mutation has been described previously, with a 3.6- to 8.3-fold decrease in susceptibility to ganciclovir (4, 11). Not surprisingly, the hybrid wild-type and C603R mutation population present in our patient behaved clinically like a ganciclovir-resistant population. Although our patient received weeks of ganciclovir and 10 days of a cidofovir analog, she had received only one intraocular injection of ganciclovir in each eye prior to developing intraocular resistance, and this mutation was not detected from plasma CMV. In addition, the DNA polymerase mutation conferring resistance to both drugs was identified only from aqueous fluid, as the plasma CMV was negative. For both instances, it should be noted that multiple populations of resistant virus

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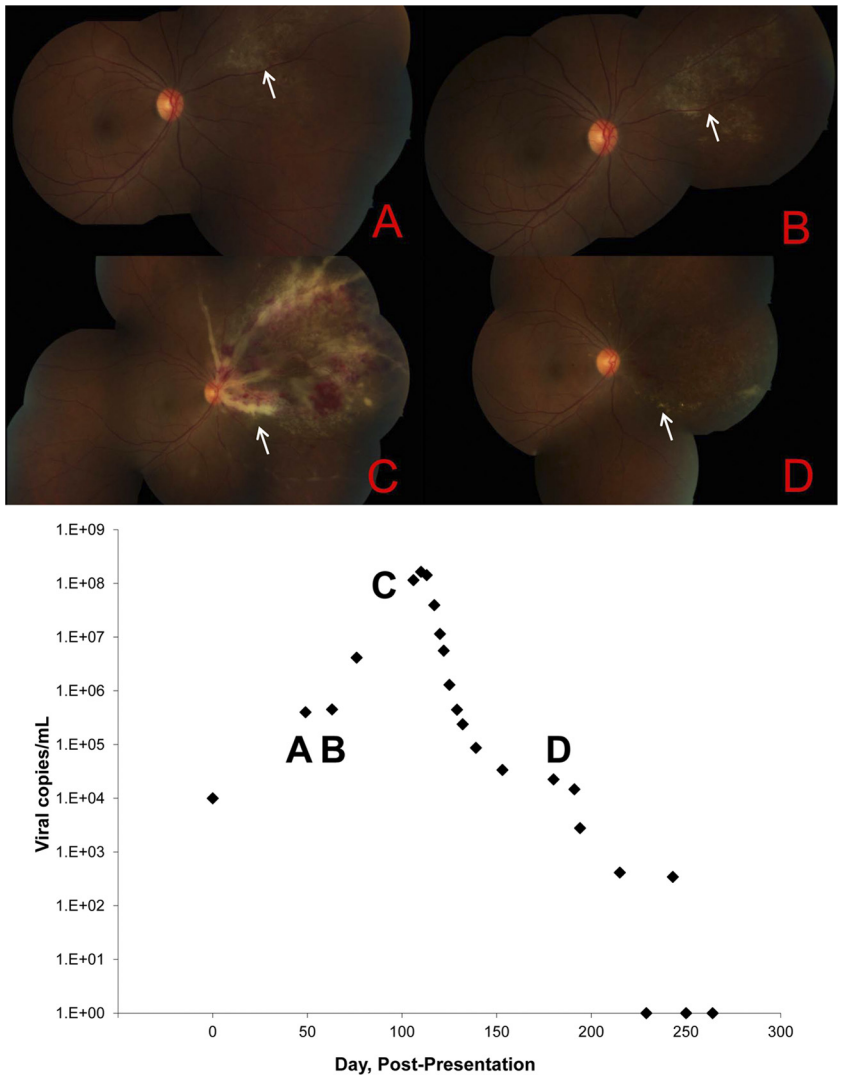

FIG 1 Right eye. (Upper panel) Images of right eye obtained using a digital fundus camera (Zeiss, Inc., Dublin, CA). After initial ganciclovir injection, the patient's clinical course was stable by day 50, with mild pigmentary changes and whitening nasal to the optic nerve (A). An initial recurrence at day 65 with increased retinal whitening (B) coincided with increased viral load, and a more severe recurrence with extensive retinal hemorrhages and whitening extending to the optic nerve at day 105 (C) was also mirrored by greatly increased viral load. Following initiation of twice-weekly ganciclovir injections, the patient's viral load decreased and her previously active retinitis evolved into a stable scar (D). (Lower panel) Quantitative viral load of aqueous fluid over time.

could have been present, with only the predominant strain being detected by the sequencing assay performed by the reference laboratory (Viracor, Lee's Summit, MO). To our knowledge there is only one report of an intraocular CMV genotype that differs from the blood genotype, and this was obtained from a vitreous biopsy specimen (14). It is likely that the frequent changes in systemic antiviral therapy contributed to the development of intraocular resistance.

For our patient, we performed quantitative real-time PCR on aqueous humor to monitor our patient's disease progression, similar to previous reports of quantitative PCR in the evaluation and monitoring of other intraocular viral infections (6, 8, 9, 13). Primers and probes used were previously described by Sanchez and Storch (12). The quantifying standard was obtained from Advanced Biotechnologies (Columbia, MD), and viral load per ml was extrapolated from the standard curve. As the volume of aqueous fluid was very limited, the fluid was diluted in nuclease-free water to obtain 200 μl for nucleic acid extraction. The dilution factor was used in the final calculation of the quantitative result. Figures 1 and 2 demonstrate that quantitative PCR performed on aqueous fluid closely mirrors the clinical progression.

The clinical diagnosis of CMV retinitis can easily be confused with other infectious processes, including acute retinal necrosis caused by herpes simplex, progressive outer retinal necrosis due to varicella zoster, and even toxoplasma chorioretinitis. Also, active progression of disease versus clinical stability and scarring can often be difficult to ascertain by clinical exam alone. Historically, vitreous biopsy has been used to definitively diagnose the intraocular pathogen in cases of chorioretinitis, but biopsy carries the risk of vitreous hemorrhage, bacterial infection, and retinal detachment. We have demonstrated that anterior chamber paracentesis, a much easier and

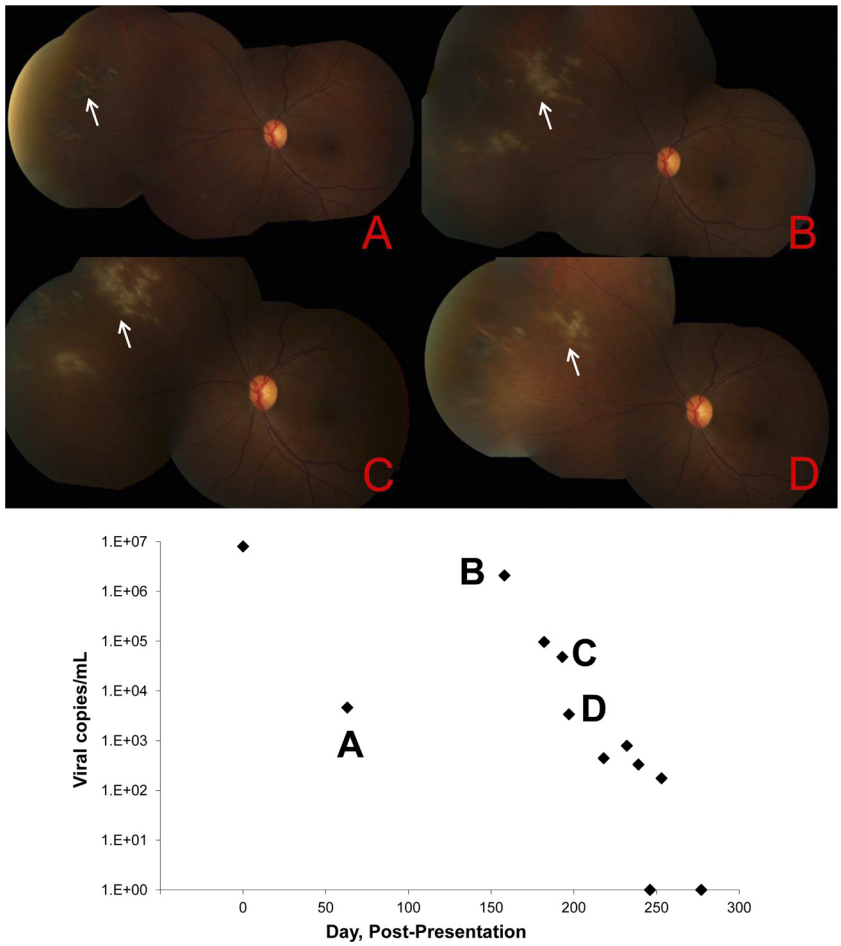

FIG 2 Left eye. (Upper panel) Images of left eye obtained using a digital fundus camera (Zeiss, Inc., Dublin, CA). After initial ganciclovir injection, the patient's clinical course was stable and she showed a residual scar by day 50 (A). She showed clinical evidence of recurrence in the left eye at day 160 (B), however, and after initiation of twice-weekly injections in the left eye, she has also shown stability without recurrence (C and D). (Lower panel) Quantitative viral load of aqueous fluid over time.

safer procedure, can be used to obtain a diagnostic specimen when the clinical exam is equivocal or when the disease seems to be progressing despite treatment. Specifically, we suggest that (i) because quantified viral loads seemed to closely mirror the clinical progression, aqueous paracentesis with quantitative PCR may be a valuable adjuvant diagnostic procedure, even in the absence of anterior segment inflammation, to determine progression in clinically equivocal scenarios and (ii) aqueous fluid can be used to obtain a genotypic profile in the evaluation of possible resistance and, because of the relative ease and low risk of collection, may be preferable to a vitreous sample (15). This case also suggests the importance of directly evaluating the intraocular fluid, as our patient's intraocular CMV resistance profile differed significantly from her systemic genotype. While currently not performed frequently in the diagnosis and treatment of CMV retinitis, the adoption of this practice may lead to both earlier and more effective treatment for chorioretinitis. Finally, we have demonstrated that repeated foscarnet injections, totaling 43 and 36 injections in our patient's right and left eyes, respectively, were effective and did not result in any frank toxicity.

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