Published in final edited form as:
Arch Ophthalmol. 2012 January ; 130(1): 113–115. doi:10.1001/archophthalmol.2011.380.

Polymerase chain reaction-based ganciclovir-resistance testing of ocular fluids for cytomegalovirus retinitis

Steven Yeh[1], **Gary Fahle**[2], **Farzin Forooghian**[3], **Lisa J. Faia**[4], **Eric D. Weichel**[5], **J. Timothy Stout**[6], **Christina J. Flaxel**[6], **Andreas K. Lauer**[6], **H. Nida Sen**[7], and **Robert B. Nussenblatt**[7]

[1]Retina Service, Emory Eye Center, Emory University School of Medicine, Atlanta, GA

[2]Department of Laboratory Medicine, National Institutes of Health, Bethesda, MD

[3]Department of Ophthalmology and Visual Sciences, University of British Columbia, Vancouver, B.C

[4]Associated Retinal Consultants, William Beaumont Hospital, Royal Oak, MI

[5]The Retina Group of Washington, Washington, D.C

[6]Casey Eye Institute, Oregon Health and Science University, Portland, OR

[7]National Eye Institute, National Institutes of Health, Bethesda, MD

Introduction

Cytomegalovirus (CMV) retinitis typically presents as a hemorrhagic, full-thickness retinitis in immunosuppressed individuals, often in the setting of HIV infection. The management of CMV retinitis includes systemic and locally administered intravitreal antivirals (i.e. foscarnet or ganciclovir), as well as the surgical intravitreal ganciclovir implant. In chronically immunosuppressed patients (i.e. transplant recipients, cancer chemotherapy) and in HIV/AIDS patients who fail to immune reconstitute, chronic CMV prophylaxis with valganciclovir may lead to ganciclovir- and foscarnet-resistant CMV strains. Moreover, the identification of drug-resistant CMV may influence the choice or dosing of antiviral medication.[1–2] Ganciclovir-resistance is classified into *genotypic* resistance defined as CMV DNA harboring a mutation known to confer antiviral resistance or *phenotypic* resistance meaning that ganciclovir at a therapeutic dose fails to exceed the concentration required to inhibit 50% of CMV growth on viral culture media. We characterize a series of patients with CMV retinitis who were evaluated for genotypic ganciclovir-resistance using PCR-based analysis of ocular fluids and describe its influence on management.

Methods

Patients diagnosed with CMV retinitis who underwent ocular PCR analysis for ganciclovir-resistance from two tertiary referral institutions (National Eye Institute, National Institutes of Health; Casey Eye Institute, Oregon Health and Science University) from June 2007

Corresponding Author: Address: Robert B. Nussenblatt, MD, MPH, National Eye Institute, National Institutes of Health, Building 10, 10N-112, Bethesda, MD 20892, DrBob@nei.nih.gov, Phone No: 301-480-3123, Fax No: 301-480-1122.

through December 2008 were reviewed. Institutional Review Board approval for medical record review was obtained from each institution.

Medical record review, ocular fluid sampling and clinical management

Demographic information and medical history including chronic immunosuppression were reviewed. Patients were treated with systemic and intravitreal antivirals at the discretion of the physician. All patients received intravitreal antiviral therapy following ocular fluid sampling. Anterior chamber paracentesis typically provided 50–100 ul of fluid; a vitreous tap yielded 200– 300 ul of fluid, which was sufficient for qualitative and quantitative PCR, and genotypic antiviral-resistance testing. Clinical management was altered depending on whether the patient showed genotypic antiviral resistance.

PCR analysis and direct CMV genome sequencing for antiviral drug resistance

The Department of Laboratory Medicine, National Institutes of Health performed all PCR analyses of ocular fluid specimens for CMV, herpes simplex virus (HSV), varicella zoster virus (VZV), and toxoplasmosis. Patients who developed CMV reactivation while on prophylactic valganciclovir therapy were evaluated for ganciclovir-resistance. Following PCR amplification of CMV DNA from aqueous fluid, direct genome sequencing of the UL97 gene encoding phosphotransferase and UL54 gene encoding DNA polymerase was performed (Supplemental methods). The gene sequences were compared to a database of polymorphisms previously defined to confer genotypic antiviral resistance.[3]

Results

Direct CMV UL97 and UL54 gene sequencing was performed from DNA extracted from ocular fluid from six patients following a reactivation of CMV retinitis. Median age was 56.5 years (range 1–73) and only two of six patients were HIV-positive (Table 1). An aqueous humor specimen was obtained from five patients and vitreous from one patient, and all samples were sufficient for PCR analyses. CMV DNA from two patients was found to contain mutations in both the UL97 and UL54 genes, prompting changes in oral and intravitreal therapy. Interestingly, both patients had been receiving valganciclovir therapy for over 12 months prior to CMV retinitis reactivation (Table 2). One patient (Patient 4) with disease reactivation who was negative for ganciclovir-resistant CMV later reported non-compliance with his antiviral medications due to tolerability issues. His retinitis eventually resolved following ganciclovir implantation. All patients received at least one intravitreal foscarnet injection and their systemic and intravitreal medications are summarized in Table 2. In total, the management of CMV retinitis reactivation was altered in 2 of 6 patients (33%) based on ganciclovir-resistance evaluation and these two patients are further described herein.

Patient 1

A 73-year-old man with large granular leukemia (LGL) with a CD4 count of 10 cells/ul, disseminated *Mycobacterium kansasii*, and previously treated CMV retinitis complained of floaters OU while on prophylactic valganciclovir (450 mg bid). Visual acuities were 20/40 OD and 20/32 OS. Slit lamp examination revealed mild anterior chamber inflammation OD.

Dilated funduscopic examination showed diffuse peripheral retinal pigment epithelium (RPE) atrophy superotemporally OD with subtle retinal whitening and hemorrhage at the atrophic RPE border.[4] Aqueous fluid PCR confirmed 1200 genome equivalents/ml of CMV DNA. Direct genome sequencing for ganciclovir-resistance was negative. Induction therapy with valganciclovir 900 mg bid was begun with resolution of the retinitis.

Eight months later, a CMV retinitis recurrence was observed, and repeated ganciclovir-resistance testing showed point mutations in the UL54 and UL97 gene, which conferred high-level genotypic resistance to ganciclovir and cidofovir (Table 2). Intravenous and intravitreal foscarnet were initiated and improvement in the retinitis was observed at 3 weeks follow-up. Unfortunately, the patient died secondary to systemic complications related to LGL.

Patient 2

A 67-year-old woman status post lung transplantation from a CMV-positive donor in 2005 for end-stage interstitial lung disease secondary to Sjogren's syndrome was on tacrolimus, mycophenolate mofetil and prednisone. Intravenous ganciclovir administered for CMV viremia was stopped after severe neutropenia developed. Oral prophylactic valganciclovir 450 mg BID was eventually initiated and filagrastim was administered.

In August 2007, she presented with floaters and a CMV retinitis reactivation posterior to a zone of peripheral RPE atrophy.[4] Aqueous fluid was positive for CMV DNA and intravitreal foscarnet was administered. Antiviral resistance testing showed UL54 and UL97 gene mutations conferring high-level ganciclovir- and cidofovir-resistance. Other UL54 gene polymorphisms were identified, but were not associated with ganciclovir-resistance (Table 1). Based on the detection of these mutations, intravitreal foscarnet injections were continued until disease resolution. Ganciclovir implantation was performed with no evidence of disease recurrence at final follow-up.

Discussion

In this series, we utilized direct CMV genome sequencing to assay for the presence of resistance mutations and demonstrated UL97 and UL54 mutations in two patients treated with greater than twelve months of prophylactic valganciclovir. The identification of drug-resistant strains of CMV led to a therapeutic change via initiation of intravitreal foscarnet with subsequent disease resolution.

Prior reports have shown that ganciclovir-resistant CMV identified using peripheral blood may be correlated with a poorer visual outcome and increased mortality risk.[1–2,5] Due to the effectiveness of highly active anti-retroviral therapy, it is possible that an increasing proportion of patients who develop CMV infections will be chronically immunosuppressed for reasons other than HIV, as observed in this series. Some of these patients may also be on chronic prophylactic valganciclovir for non-ocular CMV, potentially applying selection pressure for the emergence of resistant CMV strains. Because ganciclovir-resistance is a relative and not an absolute phenomenon, options when ganciclovir-resistance is identified

include increasing the ganciclovir dosage, implantation of a sustained-release ganciclovir intravitreal implant, switching medications, or initiating combination antiviral therapy.

Ganciclovir-resistance is not assessed in every patient with CMV retinitis because of the intensive labor and cost associated with testing. However, in selected clinical circumstances, particularly in patients with recurrent CMV retinitis while on chronic valganciclovir therapy or in refractory cases of CMV retinitis, direct gene sequencing may be helpful. Detection of mutations in CMV genome conferring antiviral resistance is commercially available through Clinical Laboratory Improvement Act-compliant reference laboratories with molecular diagnostic capabilities, sometimes within 48 to 72 hours. However, because of the small volumes typically obtained from ocular fluids and the greater degree of familiarity of reference labs with plasma, serum, and cerebrospinal fluid specimens, close communication with the reference laboratory and confirmation of their ability to process ocular specimens is recommended prior to ordering CMV drug resistance testing.

Limitations of this study include the retrospective nature of data collection and potential selection bias in the limited number of patients chosen for antiviral drug resistance testing. However, using small ocular fluid volumes, we were able to assess qualitative and quantitative CMV PCR and identify genomic mutations conferring genotypic resistance. While the utility of qualitative and real-time quantitative PCR for the assessment of viral retinitis has been established, antiviral resistance testing using ocular fluid samples may also prove to be extremely valuable in the management of ganciclovir-resistant CMV retinitis.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This research was supported by Intramural funding of the National Eye Institute, National Institutes of Health (HNS, RBN). This research was also supported by an unrestricted grant from the Research to Prevent Blindness (New York, NY) to the Casey Eye Institute, Oregon Health & Science University and Emory Eye Center, Emory University School of Medicine, Atlanta, GA. Dr. Yeh has received support from the Heed Ophthalmic Foundation and the Ronald G. Michels Fellowship Foundation.

References

1. Jabs DA, Enger C, Forman M, Dunn JP. Incidence of foscarnet resistance and cidofovir resistance in patients treated for cytomegalovirus retinitis. The Cytomegalovirus Retinitis and Viral Resistance Study Group. Antimicrob Agents Chemother. 1998; 42:2240–2244. [PubMed: 9736542]
2. Jabs DA, Martin BK, Forman MS. Mortality Associated with Resistant Cytomegalovirus among Patients with Cytomegalovirus Retinitis and AIDS. Ophthalmology. 2010; 117:128–32.5. [PubMed: 19818505]
3. Chevillotte M, von Einem J, Meier BM, Lin FM, Kestler HA, Mertens T. A new tool linking human cytomegalovirus drug resistance mutations to resistance phenotypes. Antiviral Res. 2010; 85:318–327. [PubMed: 19853628]
4. Yeh S, Forooghian F, Faia LJ, Weichel ED, Wong WT, Sen HN, Chan-Kai BT, Lauer AK, Chew EY, Nussenblatt RB. Fundus autofluorescence changes in CMV retinitis. Retina. 2010; 30:42–50. [PubMed: 19996825]

5. Jabs DA, Martin BK, Forman MS, et al. Cytomegalovirus resistance to ganciclovir and clinical outcomes of patients with cytomegalovirus retinitis. Am J Ophthalmol. 2003; 135:26–34. [PubMed: 12504693]

Table 1

Demographic information and treatment regimens

Total number of patients	6
Male (%)	4 (67)
Female	2 (33)
Median age (Range)	56.5 (10 mo–72 yrs)
HIV status	
Positive (%)	2 (33)
Negative	4 (67)
Therapy for CMV retinitis patients*	
Intravitreal foscarnet** (%)	6 (100)
Oral valganciclovir	5 (83)
IV foscarnet or ganciclovir	2 (33)
Ganciclovir implant	1 (16)

* This includes all medications administered after ocular fluid was obtained for PCR testing, including the initial foscarnet injection in all cases.

** Foscarnet administered in a dose of 2.4 mg/0.1 ml

Abbreviations HIV Human immunodeficiency virus **PCR** Polymerase chain reaction **CMV** Cytomegalovirus **VZV** Varicella zoster virus **IV** Intravenous

Table 2

Clinical details of patients undergoing ganciclovir-resistance evaluation using ocular fluid

Pt/Age/Sex	Associated medical conditions	Months of valganciclovir or ganciclovir prior to CMV reactivation†	Presence of point mutations in CMV UL97 or UL54 genes associated with ganciclovir-resistance?	CMV retinitis treatment prior to ganciclovir- resistance testing	Change in therapy?
1/73/M	Natural killer cell leukemia	> 12	Yes (9/2008) – UL97: C592G*; UL54: T503I*; No (1/2008)	Valganciclovir	Yes, intravitreal foscarnet
2/67/F	Lung transplant, mycophenolate mofetil, prednisone, tacrolimus	> 12	Yes – UL97: M460I*; UL54: A987G* UL54 mutations identified but not associated with ganciclovir-resistance: S655L, N685S, A885T, N898D	Valganciclovir	Yes, intravitreal and intravenous foscarnet
3/32/M	HIV	1	No	Valganciclovir, intravitreal foscarnet	No
4/41/M	HIV	2, poorly compliant with valganciclovir	No**	Valganciclovir, intravitreal foscarnet	No, ganciclovir implant both eyes due to poor medication compliance
5/10 mo/M	Acute lymphocytic leukemia	2	No	Intravenous foscarnet/ganciclovir, Intravitreal foscarnet	No
6/72/F	Diabetes mellitus	6	No	Valganciclovir, intravitreal foscarnet	No

† CMV reactivation was confirmed with positive CMV PCR with both qualitative and quantitive real-time PCR (data not shown). HSV, VZV, and toxoplasmosis DNA of ocular fluids were negative for all patients. Aqueous humor specimens were obtained in patients 1–5 and a vitreous specimen was obtained in patient 6.

* UL97 or UL54 point mutations associated with ganciclovir-resistance

** No UL97 mutations conferring resistance, UL54 base changes identified but none know to confer phenotypic resistance