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Detection of Ganciclovir Resistance in Patients with AIDS and Cytomegalovirus Retinitis: Correlation of Genotypic Methods with Viral Phenotype and Clinical Outcome

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Abstract

Background.—The cytomegalovirus (CMV) UL97 gene can be sequenced either from blood specimens directly amplified by polymerase chain reaction (PCR) or from culture isolates, to detect resistance to ganciclovir.

Methods.—A prospective epidemiological study was conducted in which paired specimens were routinely obtained for sequencing of the UL97 gene from blood specimens (i.e., plasma and leukocytes) directly amplified by PCR and from CMV culture isolates. The specimens then were compared with each other and in terms of results of susceptibility testing and their association with progression of retinitis.

Results.—A total of 845 paired specimens were obtained from 165 patients with AIDS and CMV retinitis. There typically was >90% agreement between the UL97 gene sequences from blood specimens directly amplified by PCR and those from culture isolates. The agreement between phenotypic resistance and the detection of UL97 mutations was >92% for PCR-amplified blood specimens and >97% for culture isolates. Plasma and leukocytes performed similarly. Progression of retinitis was correlated with the detection of UL97 mutations in PCR-amplified blood specimens, with adjusted odds ratios of 7.02 ($P = .002$) for leukocytes, 9.11 ($P = .02$) for plasma, and 17.6 for culture isolates ($P < .0001$).

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Conclusions.—Because blood specimens directly amplified by PCR can be analyzed more rapidly than can cultures (⩽48 h vs. ⩾4 weeks), sequencing the CMV UL97 gene from blood specimens directly amplified by PCR may be useful clinically.

Cytomegalovirus (CMV) infection is among the most frequently occurring opportunistic infections in patients with AIDS, and CMV retinitis accounts for ~80% of cases of CMV disease. Before the advent of highly active antiretroviral therapy (HAART), CMV retinitis affected an estimated 30% of patients with AIDS [1–5]. HAART has resulted in an 80% decrease in the incidence of CMV retinitis, but this decrease has leveled off, and new cases continue to occur [6–10]. Unless there is immune recovery, long-term, suppressive anti-CMV therapy is required to prevent a relapse of retinitis, because relapse occurs promptly after discontinuation of anti-CMV therapy for immunocompromised patients [11–13]. Although CMV retinitis may occur in patients with AIDS who are HAART naive, the majority of patients with CMV retinitis are HAART experienced, and they either have not responded to HAART or could not tolerate it [14]. Therefore, most patients with newly diagnosed CMV retinitis will require long-term, suppressive anti-CMV therapy and will be at risk for developing CMV resistance to antiviral drugs [15].

During the pre-HAART era, resistance to each of the anti-CMV drugs (ganciclovir, foscarnet, and cidofovir) was reported to occur at the rate of ~25% per person-year [16–19]. Phenotypic resistance is measured by the ability of CMV to grow in the presence of an anti-CMV drug, by use of such methods as the plaque reduction assay or a DNA hybridization assay, and it is usually expressed as the IC_{50} [20–24]. Genotypic resistance is defined by the presence of a mutation known to confer a resistant phenotype. Typically, low-level resistance of CMV to ganciclovir occurs via mutations in the CMV UL97 gene (a phosphotransferase), and high-level resistance to ganciclovir occurs via mutations in both the UL97 gene and the UL54 gene (the DNA polymerase) [25–35]. Both phenotypic and genotypic testing of culture isolates require sufficient amounts of CMV from culture specimens, which may take ⩾4 weeks to achieve and which limits the clinical utility of both types of testing.

Genotyping of CMV also may be accomplished by directly amplifying the UL97 gene from blood specimens by use of polymerase chain reaction (PCR) techniques, followed by sequencing of the gene. This may be performed in ⩽48 h, which makes it a more rapid assay for the determination of resistance of CMV, compared with either phenotypic assays or sequencing of the CMV UL97 gene from culture isolates [36, 37]. As such, this approach holds the promise of being clinically useful, because the results can be obtained sufficiently rapidly to alter therapy. For both ganciclovir and foscarnet, there is good to excellent genotype-phenotype agreement for culture isolates [25], and there is excellent genotype agreement between blood culture isolates and virus detected in vitreous specimens [38], suggesting that mutations detected in blood cultures typically are present in the eye. Because resistance to anti-CMV agents is associated with poor outcomes [39], techniques to rapidly identify patients who harbor resistant CMV are needed.

The Cytomegalovirus Retinitis and Viral Resistance (CRVR) study is a prospective cohort study of the occurrence of resistant CMV, the molecular biology of resistance, and the

clinical implications of resistance in patients with AIDS and CMV retinitis [23]. In the present study, the CRVR Study Group evaluated direct PCR amplification of blood specimens used for sequencing of the CMV UL97 gene to detect ganciclovir resistance. The results of PCR amplification and sequencing were compared with the results of sequencing of the UL97 gene from simultaneously obtained blood culture isolates and with the results of phenotypic susceptibility testing. In addition, both approaches to the identification of mutations conferring ganciclovir resistance were analyzed for their association with relapse of retinitis. Because both low- and high-level resistance to ganciclovir involve a mutation in the CMV UL97 gene, sequencing this gene alone is adequate for the detection of resistance [25, 26, 35]. Because >80% of patients with CMV retinitis are treated with some form of ganciclovir [38, 39], this approach, if successful, would have potential widespread clinical utility.

PATIENTS AND METHODS

Patients.

The CRVR study enrolled patients with both AIDS and previously untreated CMV retinitis, at 1 of 3 clinical centers: The Johns Hopkins University School of Medicine, the Northwestern University School of Medicine, and the University of Miami School of Medicine. Patients were treated for CMV retinitis in accordance with the best judgment of the clinician, and treatments were administered in a standardized fashion [23]. Patients treated with the ganciclovir implant typically also received either oral ganciclovir or valganciclovir [40, 41]. Because of the time delay in obtaining the results of virologic tests, treatment decisions were made on the basis of clinical judgment—that is, without knowledge of the results of resistance testing.

Patients were seen monthly for ophthalmologic examinations, at which time standardized fundus photographs were taken. Photographs were sent to the CRVR Fundus Photograph Reading Center at the University of Wisconsin, Madison, where CMV retinitis progression was evaluated by graders who were masked as to the results of laboratory tests and to treatment [23]. The definition of CMV retinitis progression was the standard definition used for clinical trials and epidemiologic studies of CMV retinitis—namely, the movement of a border of a CMV lesion ⩾750 μm along a front ⩾750 μm in size or the occurrence of a new lesion greater than or equal to one-quarter of a disk area in size [23, 42]. Treatment decisions were made without knowledge of the results of the Fundus Photograph Reading Center gradings.

Cultures and phenotypic susceptibility testing.

Before the initiation of therapy, blood specimens for the culture of CMV isolates were collected from all patients. Follow-up cultures were performed at 1 and 3 months after enrollment, every 3 months thereafter, and at the time of CMV retinitis progression. Culture specimens were processed locally at each clinical center, as described elsewhere [42]. All CMV isolates were tested for viral susceptibility at the virology laboratory of The Johns Hopkins Hospital, by use of either a DNA hybridization assay (Hybriwix Probe System–CMV Susceptibility Test Kit; Diagnostic Hybrids) or a plaque reduction assay [22, 23].

Previous data have demonstrated good to excellent correlation between the 2 techniques [16, 18, 23, 24]. For ganciclovir, an isolate was defined as resistant if the IC_{50} was ⩾6 μmol/L [16, 23, 24, 43, 44]. If there was no growth of CMV from a culture (i.e., if there was a negative CMV culture result), the patient was assumed to harbor a susceptible virus.

UL97 gene sequencing.

All CMV isolates recovered from blood cultures were sequenced for mutations in the CMV UL97 gene at the virology laboratory at The Johns Hopkins Hospital, as described elsewhere [25, 26, 38]. UL97 genes were amplified from culture isolates by use of PCR and were sequenced using dRhodamine or the BigDye Terminator Ready Reaction Kit (PE Applied Biosystems). The UL97 gene sequences were aligned with AD169 gene sequences by computer analysis, to determine the presence of mutations [25, 26, 38]. All identified sequence changes were known to be either mutations or polymorphisms [25, 26]; because polymorphisms do not result in phenotypic changes, only known mutations were analyzed.

PCR amplification and UL97 gene sequencing of blood specimens.

Blood specimens for direct PCR amplification and UL97 gene sequencing were obtained at the same time as culture specimens. Leukocytes (including lymphocytes, monocytes, and granulocytes) and plasma were separated, as described elsewhere [45, 46]. DNA was extracted from plasma and leukocytes, as described previously, and the CMV UL97 gene was amplified using PCR, as described elsewhere [38, 45, 46]. The amplified UL97 genes were sequenced using dRhodamine or the BigDye Terminator Ready Reaction Kit, as outlined above [38].

Analyses and statistics.

The analyses of agreement between the 2 approaches to detecting UL97 gene mutations (i.e., sequencing a culture isolate and sequencing a PCR-amplified blood specimen) were evaluated using several data sets, depending on the type of study visit, the type of specimen used, and the definition of agreement included (table 1). The purpose of evaluating multiple data sets was to avoid overstating the agreement as a result of any artifact (e.g., the large number of negative results of culture and direct PCR analysis during follow-up). Both plasma and leukocyte specimens were evaluated as sources for gene sequencing of PCR-amplified blood specimens.

In addition to analyzing all visits, we also analyzed follow-up visits only, because resistance is rare with new-onset retinitis [24, 43] and develops as the patient receives treatment [16, 47]. The specimen sets could include all specimens, only those for which there was a positive result of either culture or direct PCR, or only those for which there was a positive result of both culture and direct PCR. When all specimens were considered, a negative culture result was assumed to denote that the patient harbored a susceptible virus, as was failure to amplify the UL97 gene from a blood specimen by PCR; negative results of culture and negative results of PCR amplification of a blood specimen were considered to be in agreement.

For specimens with positive results, agreement could be defined in 2 ways. The first definition was that agreement occurred only when there was exact agreement between the UL97 gene sequences from both the culture isolate and the PCR-amplified blood specimen. Because of the presence of mixed populations of CMV and of non–resistance-conferring polymorphisms [26, 45, 46, 48, 49], it also was possible that the 2 methods could identify different gene sequences or mixtures of sequences that resulted in the same phenotype of resistance or susceptibility, and a second analysis was undertaken in which the 2 sources were considered to be in agreement if any mutation was identified.

Agreement between the 2 approaches was calculated as the percentage of agreement, and, when the definition of agreement was the presence of any mutation, the κ statistic [50]. The κ statistic adjusts for agreement expected by chance alone; $\kappa > 0.6$ is considered to denote "substantial" agreement, and $\kappa > 0.8$ is considered to denote "almost perfect" agreement. Because of the multiplicity of different gene sequences and combinations of sequences, not all sequence categories occurred with both methods; therefore, agreement based on the exact UL97 gene sequence could not be analyzed using the κ statistic.

Genotypes from culture isolates and from blood specimens directly amplified by PCR were compared for their agreement with phenotypic resistance defined by susceptibility testing of culture isolates, by use of the different data sets and statistics defined above. In these analyses, a susceptible phenotype of a culture specimen was considered to be in agreement with a genotype from either a culture isolate or a specimen directly amplified by PCR if either no UL97 gene mutation was detected or the UL97 gene could not be amplified.

The 3 sources for identification of UL97 mutations (blood culture isolates, PCR-amplified plasma specimens, and PCR-amplified blood leukocyte specimens) were evaluated for their association with retinitis progression. For this analysis, follow-up of patients was divided into 3-month intervals centered around the collection of specimens for CMV culture and direct PCR amplification and sequencing of the UL97 gene. For each 3-month interval during which ganciclovir was administered, patients were classified as having a resistant or susceptible virus on the basis of results of genotype testing [39, 51]. Because HAART-induced immune recovery may control CMV retinitis without anti-CMV therapy, HAART use during each interval was included in the model [15, 52–54]. In this analysis, HAART was defined as combination antiretroviral therapy that included either a protease inhibitor or a nonnucleoside reverse-transcriptase inhibitor. Because treatment with the ganciclovir implant results in longer times to retinitis progression than does systemic ganciclovir [40, 41], the analytic model also included ganciclovir implant use. Both crude odds ratios (ORs) and ORs adjusted for HAART use and ganciclovir implant use are reported.

RESULTS

Study population.

Data were obtained from 165 patients with AIDS and CMV retinitis who were followed prospectively. The characteristics of the study population are outlined in table 2. The demographic characteristics were similar to those reported for patients with CMV retinitis from other studies conducted during the HAART era [13, 14]. At the time of diagnosis of

CMV retinitis, 45.1% of patients were receiving HAART, and 79.4% of patients received HAART at some time during follow-up. More than 40% of patients received a ganciclovir implant as their initial therapy, and nearly two-thirds of patients received a ganciclovir implant at some time during follow-up.More than 80% of patients received some form of systemic therapy at the time of diagnosis of retinitis, with or without an implant. The rates of retinitis progression and culture resistance were 54% and 4% per person-year, respectively.

Agreement in sequencing of the UL97 gene between culture isolates and PCR-amplified blood specimens.

The agreement between UL97 mutations detected in culture isolates and those detected in PCR-amplified blood specimens is shown in table 3. A total of 845 specimen pairs were available, 737 of which were obtained during follow-up. The data sets in which there was both a culture isolate and/or a PCR-amplified blood specimen were smaller. Regardless of the data set and the definition of agreement used, the percentage of agreement always was >82% and typically was >90%. For the analysis detecting any mutation in the set of specimens in which there was both a culture isolate and a PCR-amplified blood specimen, the κ statistics were in the substantial to almost perfect range, regardless of whether all visits or only follow-up visits were analyzed. Because of the large number of negative results of culture and PCR amplification of blood specimens during follow-up (which were considered to be in agreement if both results were negative and which increased the estimate of agreement by chance alone), the κ statistics were somewhat lower for those analyses in which all specimens were included, regardless of whether all visits or only follow-up visits were analyzed. There was little difference between plasma and leukocytes as a source of PCR-amplified blood specimens for UL97 gene sequencing, when results of PCR amplification were compared with culture results.

Agreement between phenotypic susceptibility and sequencing of the UL97 gene from culture isolates and from PCR-amplified blood specimens.

Regardless of the source used for sequencing the UL97 gene (blood culture isolate, PCR-amplified plasma specimen, or PCR-amplified blood leukocyte specimen), and regardless of the data set used, phenotype-genotype agreement always was >90% and typically was >95% (table 4). However, the κ statistic, which corrects for chance agreement, indicated better agreement between phenotypic susceptibility and culture isolates as a source for UL97 gene sequencing than between phenotypic susceptibility and either plasma or blood leukocyte specimens that were directly amplified by PCR. In this analysis, sequencing of culture isolates for the UL97 gene tended to have almost perfect agreement with phenotypic measures. PCR-amplified blood leukocyte specimens for UL97 gene sequencing typically had slightly better agreement with phenotypic results than did plasma specimens, when assessed with the κ statistic.

Retinitis progression.

The associations between genotypic resistance identified from the 3 sources and retinitis progression are listed in table 5. All 3 sources for identification of a UL97 gene mutation demonstrated substantial association with retinitis progression, as is evidenced by the large ORs, both in the crude analysis and in the adjusted analysis, and, for each source, the

association was statistically significant. Nevertheless, blood culture isolates had higher ORs for retinitis progression than did either PCR-amplified plasma specimens or PCR-amplified blood leukocyte specimens. In contrast to the analyses of agreement with phenotypic resistance, in analyses of retinitis progression, PCR-amplified plasma specimens were more strongly associated with progression than were PCR-amplified blood leukocyte specimens. HAART showed no association with retinitis progression. The implant showed a reduction of ~40%–45% in the odds of progression of retinitis, but the result was not statistically significant.

DISCUSSION

Although a priori it might have been assumed that there would be excellent correlation of UL97 gene sequencing between simultaneously obtained CMV culture isolates and blood specimens for direct PCR amplification, there are technical reasons why the sources might give different results. Culture isolates require propagation of the specimen, and the virus might not grow because of technical problems, or the culture process may select for a single isolate with a growth advantage in a mixed population. Direct PCR amplification of blood specimens might (1) fail to detect low levels of virus, (2) detect only the predominant type, or (3) detect a mutation when it represents a minor population in a mixed CMV population [26, 33, 48, 49, 55]. As such, it was possible that the 2 techniques could differ, and, therefore, a comparative study was needed. Our results demonstrate substantial, but not perfect, agreement between the 2 approaches to identification of a resistant virus in the blood. Although, overall, the agreement for identification of a mutation was excellent, with agreements generally in the range of 90%–95%, there were occasional discrepancies in the actual UL97 gene sequence between culture isolates and PCR-amplified specimens of either plasma or blood leukocytes. Because the clinical goal is to identify the presence of a resistance-conferring mutation so that treatment can be changed to a drug to which the infecting CMV is susceptible [39, 56], agreement defined as the identification of any resistance-conferring mutation may have the most clinical relevance.

In a comparison of the results of direct PCR amplification of blood specimens with the results of susceptibility testing (phenotypic resistance), there was agreement in >90% of cases, but the κ statistics for PCR-amplified specimens were more modest than were those for culture isolates. This difference was more evident for PCR-amplified plasma specimens than for PCR-amplified leukocyte specimens, for which the κ statistics generally (but not always) approximated those of culture isolate sequencing and phenotypic results. Because of the occasional discrepancy between PCR-amplified blood specimens and culture isolates with respect to UL97 gene sequencing, and because the culture isolate obtained for UL97 gene sequencing was also the isolate used to measure phenotypic resistance, it is not completely surprising that the genotypes from culture isolates were better correlated with phenotypic results than were the genotypes from PCR-amplified blood specimens. The somewhat reduced κ statistics for the PCR-amplified blood specimens largely denote the potential for chance agreement in the predominance of “negative pairings” in the analysis (i.e., no culture growth and inability to amplify the blood specimen by use of PCR).

Although the agreement between these different approaches for the identification of UL97 gene mutations in the blood of patients with CMV retinitis and phenotypic results is important, a relevant clinical question is how well the various techniques associate with clinical behavior. Blood specimens (either plasma or blood leukocytes) that were directly amplified by PCR for UL97 gene sequencing were associated with retinitis progression, with substantial ORs. Although PCR-amplified blood leukocyte specimens correlated better with phenotypic resistance than did PCR-amplified plasma specimens, with regard to the prediction of clinical behavior, PCR-amplified plasma specimens performed better than did PCR-amplified blood leukocyte specimens. Hence, PCR amplification of plasma specimens for CMV UL97 gene sequencing, which is technically easier than PCR amplification of blood leukocyte specimens, may be the preferred method for clinical use. Although PCR amplification of either plasma or blood leukocyte specimens for UL97 gene sequencing correlated well with clinical behavior, it still correlated less well than did UL97 gene sequencing of blood culture isolates, for which the adjusted ORs for progression of retinitis were nearly double those for PCR-amplified plasma specimens. However, because ⩾4 weeks are required for culture of blood specimens for CMV isolates followed by phenotypic resistance testing or sequencing, whereas ⩽48 h are required for direct PCR amplification of blood specimens, the latter approach would be likely to have greater clinical utility, because clinical decisions need to be made close to the time that a specimen is obtained. Hence, the somewhat “less good” association is offset by the substantially more rapidly obtained results. Nevertheless, in situations in which direct PCR amplification of plasma specimens for sequencing of the CMV UL97 gene provides results that appear to be inconsistent with the clinical behavior of a patient receiving ganciclovir, blood culture isolates for UL97 gene sequencing may be of value, because they provide information that will correlate better with phenotypic resistance testing and with clinical behavior.

Although the present study was prospective and had a moderately large sample size, it did have limitations. The event rates for resistance and for progression were lower than those previously reported during the pre-HAART era [16], resulting in wide 95% confidence intervals around estimates and possible type II errors (e.g., for the effect of the implant on progression of retinitis). However, the detected associations for resistance had substantial ORs and were statistically significant. The discordances between phenotypic and genotypic measures of resistance and between different sources for sequencing the UL97 gene result from the limitations of the current technology and the occasional presence of mixed populations of susceptible and resistant CMV (results described elsewhere) [25, 26, 48, 49, 54, 55].

Although blood leukocytes appeared to be a better source for direct PCR amplification, when the results for blood leukocyte specimens were compared with the results obtained for culture isolates or with those of susceptibility testing, in terms of clinical behavior, PCR amplification of plasma specimens appeared to perform better. Because CMV is latent in leukocytes (including monocytes and granulocytes) [57, 58], leukocyte sources may detect smaller CMV populations, as was evidenced by the somewhat greater frequency of amplifiable CMV from the blood leukocyte source. Detection of a mutation in plasma specimens requires viral replication sufficient to have resulted in virus shed into plasma; this

suggests that, when detectable by PCR amplification of plasma, a resistant virus is likely to be present in greater amounts and may have a greater effect on clinical behavior [46].

In conclusion, these data suggest that results of PCR amplification of blood specimens for UL97 gene sequencing has reasonable agreement with results of UL97 gene sequencing from blood culture isolates and with the results of susceptibility testing. Although there are differences introduced by the technical limitations, the correlations are sufficiently good that direct PCR amplification of blood specimens appears to have clinical utility. Furthermore, there is a strong correlation with clinical behavior, suggesting clinical utility. However, the correlation with susceptibility testing and with clinical behavior is better for blood culture isolates than for PCR-amplified specimens. Therefore, despite the time involved in obtaining them, blood culture isolates may have clinical utility in selected situations, particularly those in which PCR-amplified blood specimens do not appear to correlate with clinical behavior. Finally, although blood leukocyte specimens appear to be a slightly better source than plasma specimens for agreement of the results of PCR amplification and UL97 gene sequencing with the results of culture isolates, plasma specimens appear to be a better source for predicting clinical behavior.

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Table 1.

Parameters for analytic data sets.

Category	Parameter	Comment
Visits	All	At diagnosis and follow-up
	Follow-up only	Resistance rare at diagnosis
Specimens	All	Negative results of culture and direct PCR considered to denote susceptibility
	Culture positive or direct PCR positive	
	Culture positive and direct PCR positive	
Agreement		
Between genotypes from culture and direct PCR	Exact sequence	
	Presence of a mutation	
With phenotypic resistance		Determined by susceptibility testing

NOTE. PCR, polymerase chain reaction.

Table 2.

Characteristics of the 165 patients with AIDS and cytomegalovirus (CMV) retinitis in the study population.

Characteristic, parameter	Value
Age, median (IQR), years	38 (34–43)
Sex, % of patients	
Male	64.2
Female	35.8
Race, % of patients	
White	34.0
Nonwhite	66.0
Time since AIDS diagnosis, median (IQR), months	27 (11–54)
$CD4^+$ T cell count,[a] median (IQR), cells/μL	13 (5–32)
HAART received, % of patients	
At diagnosis of CMV retinitis	45.1
Ever during follow-up	79.4
Initial CMV treatment received, % of patients	
Systemic therapy only	47.5
Ganciclovir implant only	18.6
Ganciclovir implant and systemic therapy	32.7
Ganciclovir implant received at any time, % of patients	66.1
Follow-up, mean ± SD, months	20.3 ± 27.6
Retinitis progression rate, %[b]	54
Resistance rate, %[b]	4

NOTE. HAART, highly active antiretroviral therapy; IQR, interquartile range.

[a] At diagnosis of CMV retinitis.

[b] Per person-year.

Table 3.

Comparison of UL97 mutations detected in culture isolates and in blood specimens directly amplified by polymerase chain reaction (PCR).

Visit category, specimen group, agreement definition, PCR source	Specimen pairs assessed, no.	Agreement, %	κ
All			
All specimens			
Exact sequence			
Plasma	845	97.6	Undefined
Leukocyte	845	96.8	Undefined
Any mutation			
Plasma	845	98.9	0.47
Leukocyte	845	98.1	0.52
Culture isolates or PCR-positive specimens			
Exact sequence			
Plasma	162	90.1	Undefined
Leukocyte	204	87.3	Undefined
Any mutation			
Plasma	162	96.3	0.55
Leukocyte	204	92.6	0.51
Culture isolates and PCR-positive specimens			
Exact sequence			
Plasma	86	90.7	Undefined
Leukocyte	96	90.6	Undefined
Any mutation			
Plasma	86	97.7	0.79
Leukocyte	96	97.9	0.89
Follow-up only			
All			
Exact sequence			
Plasma	737	97.7	Undefined
Leukocyte	732	96.7	Undefined
Any mutation			
Plasma	737	98.8	0.46
Leukocyte	732	97.8	0.52
Culture isolates or PCR-positive specimens			
Exact sequence			
Plasma	101	87.1	Undefined
Leukocyte	134	82.8	Undefined
Any mutation			
Plasma	101	94.1	0.54
Leukocyte	134	88.8	0.49
Culture isolates and PCR-positive specimens			

Visit category, specimen group, agreement definition, PCR source	Specimen pairs assessed, no.	Agreement, %	κ
Exact sequence			
Plasma	49	89.8	Undefined
Leukocyte	56	89.3	Undefined
Any mutation			
Plasma	49	95.9	0.78
Leukocyte	56	96.4	0.88

NOTE. PCR, polymerase chain reaction.

Table 4.

Cytomegalovirus UL97 genotype-phenotype agreement.

Visit category, specimen group, UL97 sequence source	Specimen pairs assessed, no.	Agreement, %	κ
All			
All specimens			
Culture isolate	891	99.4	0.81
PCR-positive specimen			
Plasma	845	95.6	0.33
Leukocyte	845	97.9	0.46
Culture isolates or PCR-positive specimens			
Culture isolate	275	98.5	0.84
Plasma specimen	160	95.6	0.44
Leukocyte specimen	203	92.1	0.46
Culture isolates and PCR-positive specimens			
Culture isolate	115	98.3	0.91
Plasma specimen	84	96.4	0.65
Leukocyte specimen	95	96.8	0.82
Follow-up only			
All specimens			
Culture isolate	765	99.4	0.81
PCR-positive specimen			
Plasma	737	98.4	0.33
Leukocyte	732	97.5	0.46
Culture isolates or PCR-positive specimens			
Culture isolate	181	97.8	0.83
Plasma specimen	99	92.9	0.42
Leukocyte specimen	133	94.6	0.44
Culture isolates and PCR-positive specimens			
Culture isolate	67	97.0	0.90
Plasma specimen	47	93.6	0.64
Leukocyte specimen	55	94.6	0.81

NOTE. PCR, polymerase chain reaction.

Table 5.

Risk factors for cytomegalovirus retinitis progression.

UL97 sequence source, risk factor	OR *(P)*	Adjusted OR *(P)*
Blood culture	14.9 (.0001)	
Ganciclovir resistance		17.6 (<.0001)
HAART		1.38 (.41)
Ganciclovir implant		0.56 (.16)
PCR-positive plasma specimen	8.33 (.006)	
Ganciclovir resistance		9.11 (.02)
HAART		1.28 (.53)
Ganciclovir implant		0.61 (.22)
PCR-positive leukocyte specimen	5.67 (.0004)	
Ganciclovir resistance		7.02 (.002)
HAART		1.34 (.44)
Ganciclovir implant		0.55 (.15)

NOTE. HAART, highly active antiretroviral therapy; OR, odds ratio; PCR, polymerase chain reaction.