Letters to the Editor
Limitations of Cytomegalovirus Testing

Douglas Jabs and coworkers performed a prospective study of 122 patients with cytomegalovirus (CMV) retinitis (5). They collected blood and urine CMV isolates and performed sensitivity testing of the isolates.

The interpretation of laboratory sensitivity testing of CMV strains is, however, problematic. CMV is known to be difficult to grow in cell culture, and indeed, only 60% of the cultures were positive in the study of Jabs and coworkers. A strong selection process is needed for growth in cell culture, and during this process features of the virus present in the blood or urine may be lost. Furthermore, the virus in blood or urine may be different from the virus causing the disease, in this case the virus in the retina. It is therefore questionable how representative a virus isolated in cell culture is for the disease causing virus in the retina.

The laboratory procedure for CMV sensitivity testing is less than optimal mainly because CMV shows large variation in growth in cell culture. The large variation between multiple sensitivity testing of the same isolate tested either in parallel or in sequence is rarely shown in scientific articles.

It is therefore not surprising that sensitivity testing results may vary between different laboratories where procedures may differ. The average 50% inhibitory concentration (IC50) of CMV isolates obtained from untreated patients may vary between different laboratories by a factor of 2 (2).

The clinical relevance of CMV sensitivity testing is subject to much debate due to factors mentioned above and due to the limitation that sensitivity testing can be performed only for patients with positive blood or urine cultures. A positive and ganciclovir-sensitive blood or urine culture during adequate CMV treatment is itself associated with poor prognosis measured as subsequent contralateral eye disease (odds ratio, 3.82) (3). Jabs and coworkers have showed an association between resistance against ganciclovir and subsequent contralateral eye disease, while other studies have failed to detect an association with progression of CMV retinitis (for a review, see reference 2).

Of crucial importance for all laboratories is to carefully define their cutoff levels for CMV sensitivity testing. This is not a trivial problem, and the cutoff levels should be carefully scientifically motivated and the above-mentioned uncertainties should be accounted for. Ganciclovir has attracted most attention, and Drew and coworkers have proposed two cutoff levels (reviewed in reference 2). They considered IC50s above 12 μM as indicating resistance and values below 6 μM as indicating sensitivity, while intermediate values were considered to indicate “intermediate susceptibility.” Jabs and coworkers used 6 μM as cutoff for resistance (3). The rationale for the selection of this level is, however, unclear. It is obvious, due to the uncertainties, that each laboratory needs to carefully assess CMV isolates obtained from untreated patients in order to ensure that the cutoff limits are set in an unbiased way. This is especially important for direct comparisons between different treatments.

Jabs and coworkers performed a statistical comparison between the relative risk for foscarnet resistance during foscarnet treatment and the relative risk for ganciclovir resistance during ganciclovir treatment (Table 3 in reference 5). This type of comparison is very much dependent on factors listed above but also and in particular on the patient population and the selected cutoff levels. The study performed by Jabs et al. was not randomized or blinded. The treatment for each patient was assigned by their physician according to the clinical presentation of the disease. The influence of this design on the foscarnet-ganciclovir comparison is unknown, but the introduction of a bias probably cannot be excluded. The cutoff levels were unfortunately not justified, in contrast to previous work (1, 4).

Previous work by the same authors gives us the possibility to speculate (4). The authors had earlier reported IC50s for CMV isolates obtained from untreated patients (4). It is important to note that the cutoff used in the recent study for ganciclovir resistance (6 μM) is 3.5 standard deviations (SD) higher than the average IC50 (1.31 ± 1.34 μM) for blood isolates (2.6 SD higher than that [1.79 ± 1.65 μM] for urine isolates) while the cutoff used for foscarnet resistance (400 μM) is only 1.6 SD higher (IC50, 209 ± 117 μM) (1.4 SD higher for urine isolates [IC50, 214 ± 133 μM]) (Table 4 in reference 5). These data thus indicate that the cutoff used in the recent study for ganciclovir resistance may be further away from the average IC50 for the virus isolates for untreated patients than the cutoff for foscarnet. If true, this would bias any comparison between the relative risks for ganciclovir and foscarnet resistance. It is therefore very important that Jabs and coworkers clearly show the scientific background for the selection of the cutoff levels and the justification for the comparison of the relative risks for ganciclovir and foscarnet resistance.

REFERENCES
Author’s Reply

We appreciate the comments of Drs. Harmenberg and Brytting on the difficulties encountered in defining laboratory resistance and understanding its relationship to clinical outcomes. It is precisely for the reasons they stated that we have undertaken the prospective epidemiological study outlined in our articles (2, 3). Cultures for CMV are not always positive even in untreated patients. Our estimates of resistance assume that culture-negative patients harbor sensitive virus. This assumption is a conservative one and makes our estimates of the probability of resistance minimum estimates. Problems with interlaboratory variability do exist. However, we have stressed quality control throughout development of this study, continually compare the Hybriwix assay with the plaque reduction assay, and have found a relatively good correlation, particularly for ganciclovir and foscarnet.

We agree that the threshold for considering an isolate resistant is critical. For ganciclovir and foscarnet we have used a threshold that is above the 95th percentile value for IC50 among untreated patients. For foscarnet the 95th percentile value for IC50 was 387 μM among untreated patients. For ganciclovir it was 5.45 μM, providing a rationale for the 400 μM cutoff for foscarnet and 6 μM cutoff for ganciclovir. This approach was used by Drew et al. (1), who arrived at similar thresholds. However, because of the uncertainty of the foscarnet threshold, we also presented data using a threshold of 500 μM. Even with that threshold, there was still a substantial incidence of foscarnet resistance (19% at 9 months). Ultimately, the issue will be determined by whether resistance-conferring mutations can be identified in patients who harbor virus with an IC50 above a specific threshold. Previous data have suggested that low-level ganciclovir resistance (IC50 > 6 μM) is associated with mutations in the CMV UL97 gene and that high-level resistance (generally, IC50 > 12 μM) is associated with mutations in both the UL97 gene and the UL54 (DNA polymerase) gene (4). Little information concerning phenotype-genotype correlation is available for foscarnet. We have begun to evaluate our specimens for ganciclovir resistance and foscarnet resistance-conferring mutations, and although the data are preliminary, they tend to confirm our initially chosen thresholds.

We also agree that the CMV present in the eye is the one causing disease and may be different than the one isolated from another compartment. One of our goals is to determine whether isolates identified from the blood and/or urine, compartments which are easily accessible, correlate with the behavior of ocular disease. To that end we are collecting clinical data and collecting specimens from the eye and blood at the time of vitreoretinal surgery to compare CMV genotypes.

We believe that our data represent the best available data to date. We recognize limitations inherent in any study of this type. Ultimately, the detection of resistance-conferring mutations and correlation with clinical behavior will permit refinement of our estimates.

REFERENCES


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