Molecular methods have had a significant impact on the diagnosis of viral infections because of their superior sensitivity and rapid turnaround time compared with conventional diagnostic methods. These characteristics have allowed molecular tests to play a central role in the use of testing protocols for managing viral infections. Several examples of such protocols are reviewed in this report, including the use of molecular testing for early disease detection to improve overall disease management and to direct antiviral therapy.

Historically, algorithms or testing protocols have not been commonly used in the diagnosis and management of viral infections. This is, in part, because of the slow turnaround time of standard viral culture methods. Only relatively recently have rapid tests that detect viral antigens or nucleic acid come into clinical use. Molecular methods have had a significant impact on the diagnosis and management of viral infections because they are rapid, reproducible, and sensitive. In addition, the use of viral load testing has revolutionized the management of some viral infections, most notably HIV-1. This report will present approaches to using protocols for the management of viral infections. Three specific examples are discussed in detail, all of which use molecular methods: (a) early disease detection, the specific example being the use of molecular testing to guide preemptive therapy for cytomegalovirus (CMV) infection in solid organ transplant recipients; (b) improving overall disease management through the use of HIV-1 genotyping to guide antiretroviral therapy; and (c) viral testing to direct therapy, via HCV genotyping to determine duration of antiviral therapy.

Early Disease Detection

MOLECULAR TESTING TO GUIDE PREEMPTIVE THERAPY FOR CMV INFECTION IN SOLID ORGAN TRANSPLANT RECIPIENTS

CMV is a member of the herpesviridae family of viruses. Infection is common, with 40–100% of adults being seropositive. The two periods of increased infection occur in the perinatal period and during the reproductive years (1). After primary infection, CMV will establish a life-long latent infection. Reactivation of infection can occur in immunocompromised individuals, and for this reason, CMV infection is a major infectious complication in solid organ transplant recipients (2). The clinical presentation of CMV infection in these patients includes febrile syndromes, retinitis, pneumonitis, esophagitis, and colitis (2). In addition to reactivation of latent infection, primary CMV infection may occur in solid organ transplant recipients. Primary infections that occur when patients are immunosuppressed tend to be more severe than reactivation of latent infections.

A key diagnostic issue for CMV infection is distinguishing active disease from latent infection. The goals are to identify patients at risk for disease and to give preemptive therapy to prevent the development of CMV disease. This approach to using preemptive therapy relies on the availability of a test that can predict the risk of developing CMV disease. Quantitative molecular assays have been developed to determine whether CMV viral load concentrations are useful in differentiating latent infection from active disease. Two such assays are the hybrid capture assay (Digene Corp.) and Amplicor CMV Monitor Test (Roche Molecular Systems).

An important study of the clinical utility of CMV viral load was conducted on liver transplant recipients (3). This study monitored 97 liver transplant recipients for 12 weeks after transplant with weekly quantitative CMV DNA PCR (Amplicor Monitor) testing. High-risk individuals (donor organ CMV seropositive and recipient CMV seronegative) were treated for 12 weeks with ganciclovir

1 Nonstandard abbreviations: CMV, cytomegalovirus; HCV, hepatitis C virus; IFN, α-2b interferon; GART, genotypic antiretroviral-resistance testing; No-GART, non-GART; and HCV, hepatitis C virus.
and then monitored for an additional 8 weeks. No preemptive therapy was used in the study. Of the 97 individuals enrolled, 61 developed CMV infection, and 21 developed CMV disease. CMV infection was defined as a positive laboratory test (CMV culture, antigenemia, or PCR), whereas CMV disease was defined as end organ involvement with culture or histologic evidence of invasive CMV.

With the use of a positive cutoff of >400 copies/mL of plasma, PCR had a sensitivity of 100%, a specificity of 47%, a positive predictive value of 34%, and a negative predictive value of 100%. Different viral load cutoffs were analyzed using ROC curves. As the viral load cutoff increased, the specificity increased, but the sensitivity decreased. The optimal viral load cutoff predicting disease was 2000–5000 copies/mL of plasma (for >2000 copies/mL, sensitivity, 91%; specificity, 75%; positive predictive value, 50%; negative predictive value, 99.6%) (3).

A similar study was conducted with 52 renal transplant recipients using the hybrid capture assay (4). The risk of CMV disease after renal transplant increased from 1.5% with a viral load of 10^3 copies/mL to 73% with a viral load of 10^5 copies/mL. Notably, the viral load values between the Amplicor and hybrid capture assays did not correlate. The molecular methods are different and the Amplicor assay measured viral load in plasma, whereas the hybrid capture assay measured viral load in whole blood.

Sia et al. (5) evaluated the use of viral load in predicting relapsing CMV infection. Solid organ transplant recipients (n = 24) with CMV infections were treated with ganciclovir for 2 weeks. CMV viral load was measured before and after therapy. Eight of the 24 individuals developed relapsing infection. Persistently detectable viral load after 2 weeks of therapy was associated with relapsing CMV infection (5). Moreover, in a separate study, patients with documented ganciclovir resistance had persistently increased CMV viral load concentrations during therapy (20,000–70,000 copies/mL by the Amplicor assay) (6).

On the basis of the studies described above, it is possible to use a testing protocol to monitor solid organ transplant recipients for the development of CMV disease. Monitoring patients weekly after transplant with a quantitative CMV assay can be used to guide preemptive therapy for CMV infection. When preemptive therapy should be initiated will depend on the assay used, as well as the type of organ transplant. In addition, monitoring viral load after completion of therapy may be useful in predicting relapsing disease.

**Improving Overall Disease Management**

**HIV-1 Genotyping to Guide Antiretroviral Therapy**

Viral load testing has revolutionized the management of antiretroviral therapy in individuals with HIV-1 infection. Viral load concentrations are used to determine when to initiate antiretroviral therapy, and they are used to monitor patient response to antiretroviral therapy (7). The current standard of care is to treat individuals with combinations of antiretroviral agents and to monitor viral load during therapy. A baseline viral load is obtained before initiating therapy. Viral load is routinely measured 1 month after initiating or changing therapy; it is then monitored monthly until the goal of therapy is reached and every 3 to 4 months thereafter. Testing is performed more frequently if there is a clinical indication (7). The goal is an undetectable viral load, which, depending on the assay, would be <400 copies/mL of plasma or <50 copies/mL of plasma. Failure of antiretroviral therapy refers to the inability to achieve or maintain viral suppression. Causes of treatment failure include development of resistance, nonadherence, and inadequate drug concentrations (8).

HIV-resistance testing can be useful in managing individuals for whom antiretroviral therapy has failed. Several studies evaluating the clinical utility of genotypic testing are reviewed below; however, a summary of studies evaluating phenotypic testing is beyond the scope of this discussion. Methods for genotypic testing include automated sequencing, DNA chip-hybridization assays, and the line-probe assay. Currently, most HIV-1 genotyping for clinical use is performed by the use of automated sequencing methods. The protease gene and the majority of the RT gene are sequenced to identify resistance mutations.

Several trials have established the clinical utility of genotypic assays in the management of individuals with HIV-1 infection (9, 10). The VIRADAPT study was a prospective, open, randomized, and controlled study of individuals with HIV-1 infection for whom combination therapy failed (9). Subsequent antiretroviral therapy was managed either by standard of care (control group; n = 43) or on the basis of genotyping results (genotype group; n = 65). At 3 and 6 months after enrollment, the genotype group had a greater decrease in viral load than the control group. The decrease in viral load at 6 months was 1.15 log_{10} for the genotype group vs 0.67 log_{10} for the control group. In addition, at 6 months, 32% of individuals in the genotype group had viral load concentrations <200 copies/mL, whereas 14% of the control group had viral load concentrations <200 copies/mL. After 6 months, individuals in the control arm were offered open-labeled genotypic assays. A benefit was observed in the control group, for which the percentage of individuals achieving an undetectable viral load (<200 copies/mL) increased from 14% at 6 months to 25.7% at 12 months. In addition, the benefit observed in the genotype arm persisted at 12 months (11).

A second prospective randomized controlled trial of antiretroviral management on the basis of genotyping was conducted with patients for whom therapy had failed (10). Individuals with a threefold or greater increase in
viral load after at least 16 weeks of combination antiretroviral therapy were randomized to either the experimental group [genotypic antiretroviral-resistance testing (GART); n = 78], or the control, non-GART group (No-GART; n = 75) group. In the GART group, genotyping was performed and interpretations of the results and suggested regimens were provided to clinicians. In the No-GART group, treatment choices were made without genotyping information. For the GART group, the mean viral load at 4 and 8 weeks after enrollment decreased by 1.19 log_{10}. The No-GART group had decreases in viral load of 0.61 log_{10} during the same time period (P = 0.0001) (10). The best virologic responses occurred in patients who received three or more drugs to which their HIV-1 appeared susceptible. In the study, patients for whom triple-drug therapy had failed responded better when their subsequent regimen was chosen on the basis of genotyping with expert advice compared with those without genotyping (10).

A testing protocol for monitoring individuals with HIV-1 infection during therapy would begin with monitoring viral load. Once there is evidence of virologic failure, there are studies supporting the use of resistance testing to assist in determining the duration of therapy. These three examples demonstrate the clinical utility of molecular testing protocols in virology.

In summary, there is clinical utility for the diagnosis and management of CMV, HIV, and HCV by molecular methods. Quantitative PCR determination of CMV viral load in solid organ transplant recipients can predict CMV disease and relapse. Viral load testing in patients with HIV infection is currently used to monitor the efficacy of treatment. Additionally, studies have shown the benefit of using genotypic assessment to manage antiretroviral therapy. Furthermore, there is evidence that HCV genotyping is useful for determining the duration of IFN–ribavirin therapy. These three examples demonstrate the clinical utility of molecular testing protocols in virology.

References


